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BIOLOGICAL VALUES OF WHEAT AND ALMOND NITROGEN.

By AGNES FAY MORGAN and ALICE M. HEINZ.

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(Received for publication, November 13, 1918.)

The attempt to compare the biological values of various protein foods, made by Karl Thomas (1) in 1909, has resulted in a good deal of discussion, but few confirming experiments. The brevity of his nitrogen balance periods, as well as the arbitrary formulas used by him in the calculation of the percentage efficiencies reported, are the basis of criticism made recently by American authors (2, 3).

The comparison of minimal quantities of food nitrogen which suffice to maintain equilibrium in the adult body may, however, be accepted as indicating to some degree the relative protein values of those foods. The application of data obtained in this way may need to be carried out with caution nevertheless, since as McCollum, Simmonds, and Parsons (4) have pointed out, the nitrogen metabolism in the adult body may differ in some respects from that observed during the growth of the young, or during the process of lactation.

EXPERIMENTAL.

Two foods, wheat gluten and almond meal, were used by us in a double series of modified repetitions of nitrogen equilibrium experiments. These foods were chosen because they were thought to be relatively pure protein mixtures easily freed from the other constituents usually present in the natural foods, and might therefore be calculated as furnishing a definite quantity of absorbed amino-acid-yielding substances.

The procedure was in part that used by Steck (5), and in part that followed by Thomas (1). Six experiments were carried out,

each consisting of a fore-period of 3 days of protein-poor diet, 2 days of the protein diet, and an after-period of 1 day of protein-poor diet.

The relatively protein-free basal ration used in the fore- and after-periods, as well as with the protein in the experimental period, was of two kinds, the first rich in total carbohydrates, but poor in cellulose, the second of the same total carbohydrate con-

TABLE I.

Basal Rations.

			Nitrogen.	Calories.
		gm.	gm.	
Basal Ration I. (1st day of first gluten experiment.)	Prunes	100.5	0.33	303.5
	Cream	230.0	0.92	448.5
	Sugar	80.0		320.0
	Tapioca	115.0	0.07	411.8
	Bananas	242.0	0.50	239.5
	Butter	10.0	0.01	76.9
	Total		1.83	1,800.2
Basal Ration II. (3rd day of almond experiment.)	Swedish health bread (a rye hardtack)	91.0	1.35	317.1
	Bananas	85.0	0.17	84.1
	Sugar	46.0		184.0
	Lettuce	75.0	0.12	12.0
	Olive oil	11.1		99.9
	Apples	246.0	0.15	154.8
	Celery	73.0	0.10	10.9
	Butter	75.0	0.12	576.7
	Cream	146.0	0.58	284.7
	Honey	65.8	0.04	219.4
	Total		2.63	1,943.6

tent, but poor in cellulose. The nitrogen content of these diets varied little as will be seen from Table I, but the effect of the difference in composition upon the availability of proteins is marked.

No attempt was made to ingest precisely the same quantity of these rations each day of the experiments, but the totals taken varied in general very little from the examples given in Table I.

The subject was a healthy young woman of 59 kilos body weight, whose weight did not change throughout the experimental period of nearly 6 months.

For the gluten experiments Sanitarium gluten sticks were used, manufactured by the Sanitarium Food Company, St. Helena, California. The almond meal was prepared by chopping the blanched nuts very fine, sifting them, and washing five or six times with ether in order to decrease the fat content. The gluten was 6.80 per cent nitrogen; the almond meal of three different preparations, 6.28, 4.60, 4.14 per cent, respectively.

TABLE II.

Balances with Gluten Nitrogen.

Basal Diet I.

Day of experiment.	N ingested.	N excreted.			Balance.	Added N ingested per kg. body wt.
		Urine.	Feces.	Total.		
Experiment 1.						
	gm.	*gm.*	*gm.*	*gm.*		*gm.*
1	1.83					
2	2.24	3.79	0.61	4.40	−2.16	
3	1.86	3.59	0.69	4.28	−2.42	
Gluten period. 4	6.05	5.12	0.89	6.01	+0.04	0.102
Gluten period. 5	6.13	5.59	0.20	5.79	+0.34	0.102
6	1.86	4.33	—	4.33	−2.47(?)	
Experiment 2.						
1	1.60					
2	1.89	4.94	0.87	5.81	−3.92	
3	1.36	3.46	0.87	4.33	−2.97	
Gluten period. 4	4.52	3.10	0.65	3.75	+0.77	0.077
Gluten period. 5	4.71	5.15	0.48	5.63	−0.92	0.077
6	1.40	2.87	0.48	3.35	−1.95	
Basal Diet II.						
1	2.56					
2	2.25	4.73	0.77	5.50	−3.25	
3	2.64	3.78	0.37	4.15	−1.51	
Gluten period. 4	3.98	4.80	0.35	5.15	−1.17	0.068
Gluten period. 5	4.07	6.11	0.33	6.44	−2.37	0.068
6	2.28	4.50	0.33	4.83	−2.55	

The urine and feces were collected during 5 days of the experiment, the 1st day being left out of account. Nitrogen determinations were made at once upon the fresh samples. The amount of nitrogen added in gluten or almond meal during the 2 day protein period was determined by the minimum nitrogen excretion on the preceding carbohydrate-rich diet (Tables II and V).

Biological Value of Gluten Nitrogen.

If the biological value of the gluten nitrogen is calculated from these results according to the formulas proposed by Thomas (1), the following results are obtained (Table III):

Formula A.

$$\frac{\text{Urine of N-free diet + balance}}{\text{N absorbed}} = \text{biological value.}$$

Formula B.

$$\frac{\text{Urine of N-free diet + fecal N + balance}}{\text{N ingested}} = \text{biological value.}$$

TABLE III.

Biological Value of Gluten (Wheat) Nitrogen.

Basal diet.	Experiment No.	Protein day.	Biological value.	
			A	B
I	1	1	70.5	74.8
		2	66.4	67.4
I	2	1	109.5	108.0
		2	60.2	62.1
II	1	1	72.1	74.6
		2	38.1	42.8
Average			69.4	71.6 70.5
Thomas' result with wheat flour (average)			37.99	42.43

If we omit from the averages for gluten the figures which strikingly deviate from the rest, those for the 1st day of the second experiment, Diet I, and the 2nd day with Diet II, the averages become 67.3 and 69.7.

The per cent of total nitrogen of the diet furnished by the gluten was as shown in Table IV.

TABLE IV.

Experiment No.	Day of experiment.	Total N of diet.	Total gluten N.	Total N from gluten.
		gm.	*gm.*	*per cent*
1 (Diet I)	4	6.05	4.50	74.4
	5	6.12	4.50	73.5
2 (Diet I)	4	4.52	2.98	65.9
	5	4.71	2.98	65.7
Diet II	4	3.98	2.27	57.0
	5	4.07	2.27	55.8
Average				65.4

TABLE V.

Balances with Almond Nitrogen.

Basal Diet I.

Day of experiment.	N ingested.	N excreted.			Balance.	Added N ingested per kg. body wt.
		Urine.	Feces.	Total.		
Experiment 1.						
	gm.	*gm.*	*gm.*	*gm.*		*gm.*
1	1.62					
2	1.78	4.84	0.38	5.22	−3.44	
3	1.51	2.48	0.33	2.81	−1.30	
Almond 4	3.25	3.64	—	3.64	−0.39(?)	0.065
period. 5	1.36	3.95	—	3.95	−2.59(?)	
Experiment 2.						
1	1.91					
2	1.98	4.04	0.86	4.90	−2.92	
3	1.65	3.74	1.05	3.79	−3.14	
Almond 4	4.44	3.01	1.07	4.08	+0.36	0.071
period. 5	3.96	4.31	1.07	5.38	−1.42	0.071
6	1.39	4.29	1.07	5.36	−3.97	

Basal Diet II.

1	2.12					
2	2.74	4.51	1.29	5.80	−3.06	
3	2.66	3.58	0.29	3.87	−1.21	
Almond 4	3.53	3.63	0.50	4.13	−0.60	0.065
period. 5	4.22	4.77	0.50	5.27	−1.05	0.065
6	2.22	3.67	0.50	4.17	−1.95	

Biological Value of Almond Nitrogen.

If we omit from the averages in Table VI the strikingly unusual figures obtained for the 2nd day of the first experiment, the averages become 86.7 and 89. This indicated high value of the nut protein is in accord with the results reported by Hoobler (6) on the value of nut proteins for human milk production.

TABLE VI.

Biological Value of Almond Nitrogen.

Basal diet.	Experiment No.	Day.	Biological value.	
			A	B
I	1	1	100.0	100.0
		2	121.3	116.6
	2	1	—	—
		2	80.2	85.6
II		1	98.3	98.3
		2	68.2	72.0
Average			93.6	94.5
			94.0	

The per cent of the total nitrogen of the diet furnished by the almond meal is shown in Table VII.

TABLE VII.

Experiment No.	Day of experiment.	Total N of diet.	Total almond N.	Total N from almond.
		gm.	*gm.*	*per cent*
1 (Diet I)	4	3.25	1.75	53.8
2 (Diet I)	4	4.43	2.98	67.4
	5	3.96	2.98	75.2
Diet II	4	3.53	1.97	55.8
	5	4.21	1.97	46.8
Average				60.0

Effect of Change in Basal Diet.

It is interesting to note that the biological values of both protein foods vary considerably in the two diets. The difference can hardly be ascribed altogether to changes in per cent absorbed, due to different proportions of crude fiber in the basal ration, as will be seen from Table VIII.

TABLE VIII.

Absorption of Gluten and Almond Nitrogen.

Diet.	Experiment No.	Day.	N ingested.	N in feces.	N absorbed.	
			gm.	gm.	gm.	per cent
			Gluten.			
I	1	1	6.06	0.89	5.17	85.3
		2	6.13	0.20	5.93	96.5
	2	1	4.52	0.65	3.87	85.6
		2	4.71	0.48	4.23	89.8
Average						89.3
II		1	3.98	0.35	3.63	91.0
		2	4.07	0.33	3.74	91.8
Average						91.4
			Almond.			
I	2	1	4.44	1.07	3.37	78.1
		2	3.96	1.07	2.89	73.2
Average						75.6
II		1	3.53	0.50	3.03	85.9
		2	4.22	0.50	3.72	88.2
Average						87.0

Since the basal diets are not free of protein, and since the kind of protein material varies considerably in the two, a variation in its value as a supplemental source of nitrogen may well be assumed. The results recorded by Hart and Humphrey (7) in

their cattle feeding experiments with varying basal rations, indicating the efficiencies for milk production of certain protein concentrates, show similar divergences. They emphasize repeatedly the limitations of any classification of natural foods in respect to the efficiency of their proteins based on experiments involving a single food material or a single food mixture.

SUMMARY.

1. The "biological values" of wheat gluten and almond meal, determined by the usual minimum nitrogen feeding method and calculated according to the formulas proposed by Thomas, are found to average 70.5 and 94.0. This figure for wheat gluten is nearly twice that found by Thomas, using wheat flour.

2. When as much as 0.102 gm. of gluten per kilo of body weight was fed, a positive nitrogen balance was obtained, but when the intake fell to 0.077 gm. per kilo, the positive balance was not maintained, and at 0.068 gm. per kilo became negative.

3. A satisfactory positive nitrogen balance was not obtained with the largest amount of almond meal used, 0.071 gm. per kilo of body weight.

4. The character of the basal low protein diet used affected the apparent availability of these protein foods, as expressed in terms of the Thomas formulas for biological values.

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A METHOD OF EXPRESSING NUMERICALLY THE GROWTH-PROMOTING VALUE OF PROTEINS.*

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(Received for publication, December 7, 1918.)

A young animal continuously increases its food intake during growth, hence two which grow at different rates eat unlike quantities of food. We have already pointed out[1] that comparisons of the value of different proteins for growth can be made when the animals eat the same amount of food in the same number of days and gain the same amount of weight, the protein factor being the only variable. As it is exceedingly difficult to conduct experiments which fulfil these requirements we have sought to develop a method whereby the relative values of proteins for growth can be expressed numerically.

Since food intake is quite closely regulated by the calorific requirements of the animal approximately as much food is eaten under otherwise similar conditions whether this contains a high or low percentage of protein. If a single series of experiments is made with diets containing a given percentage of protein it is obviously impossible to demonstrate the *maximum* power of any protein to promote growth. When the proportion of protein in the food is so restricted that the protein factor alone determines the rate of growth, it should be possible to find the concentration which promotes the greatest gain of body weight relative to the protein ingested by supplying foods containing *different* percentages of protein. However, this is possible only within

* The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

[1] Cf. Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1916, xxvi, 1.

limits, for differences in physical activities and the inherent capacity of the animal to grow may affect the amount of growth made within a fixed time. Consequently we cannot expect that,

TABLE I.

Source of protein.	Protein in food.	Rat.	Initial body weight.	Gain in 4 wks.	Total intake.		Intake per gm. of gain.		Gain per gm. of food.	Gain per gm. of protein.
					Food.	Protein.	Food.	Protein.		
	per cent		*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Lactalbumin.	16.2	2920♂	62	64	208	33.2	3.3	0.52	0.31	1.93
		2924♂	61	74	192	30.7	2.6	0.42	0.39	2.41
		1810♂	73	60	202	32.3	3.4	0.54	0.30	1.86
		1812♂	67	45	169	27.0	3.8	0.60	0.27	1.67
Average				61	198	30.8	3.3	0.52	0.32	1.97
Lactalbumin.	10.3	2474♂	71	60	213	21.9	3.6	0.37	0.28	2.74
		2931♂	63	47	189	19.5	4.0	0.42	0.25	2.41
Average				54	201	20.7	3.8	0.40	0.27	2.58
Lactalbumin.	7.9	2625♂	64	62	250	19.5	4.0	0.31	0.25	3.18
		2631♂	70	56	254	19.8	4.5	0.35	0.22	2.83
Average				59	252	19.7	4.3	0.33	0.24	3.01
Lactalbumin.	6.2	2596♂	66	31	194	12.0	6.3	0.39	0.16	2.58
		2606♂	67	39	233	14.5	6.0	0.37	0.17	2.69
Average				35	214	13.3	6.2	0.38	0.17	2.64
Lactalbumin.	4.9	2044♂	63	18	154	7.5	8.6	0.42	0.12	2.40
		2049♀	63	19	168	8.2	8.9	0.43	0.11	2.32
Average				19	161	7.9	8.8	0.43	0.12	2.36
Lactalbumin.	3.3	2201♂	68	7	131	4.3	18.7	0.62	0.05	1.63
		2203♂	67	7	172	5.7	24.6	0.82	0.04	1.23
Average				7	152	5.0	21.7	0.72	0.05	1.43

on a given diet, each animal will make quite the same gain of weight during equal intervals of time. How great these individual variations may be is illustrated by the data given in Tables I and II. From these we see that while the *absolute*

gains of body weight may differ by even 75 per cent, the differences between the gains in body weight *per gm. of protein eaten* are very much less, though by no means inconsiderable. It is consequently necessary to employ a larger number of animals

TABLE II.

Source of protein.	Protein in food.	Rat.	Initial body weight.	Gain in 4 wks.	Total intake.		Intake per gm. of gain.		Gain per gm. of food.	Gain per gm. of protein.
					Food.	Protein.	Food.	Protein.		
	per cent		*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Casein.	17.4	1592♂	62	54	167	29.0	3.1	0.54	0.32	1.86
		1599♂	76	49	198	34.4	4.0	0.70	0.25	1.43
		1617♂	61	74	225	39.1	3.0	0.53	0.33	1.89
		1618♂	73	63	220	38.2	3.5	0.61	0.29	1.65
		1634♂	58	53	206	35.8	3.9	0.68	0.26	1.48
		1652♂	66	59	181	31.5	3.1	0.53	0.33	1.87
		1655♂	60	43	151	26.2	3.5	0.61	0.28	1.64
		1657♂	65	55	164	28.5	3.0	0.52	0.34	1.93
		1689♂	63	50	170	29.6	3.4	0.59	0.29	1.69
Average				55.5	187	32.5	3.4	0.59	0.30	1.74
Casein.	14.7	2465♂	77	50	203	29.8	4.1	0.60	0.25	1.68
		2619♂	64	69	238	35.0	3.5	0.51	0.29	1.97
		2620♂	65	83	246	36.2	3.0	0.44	0.34	2.29
Average				67	229	33.7	3.5	0.52	0.29	1.98
Casein.	12.0	2117♂	69	38	159	19.1	4.2	0.50	0.24	1.99
		2623♂	65	62	217	26.0	3.5	0.42	0.29	2.38
		2630♂	63	67	233	28.0	3.5	0.42	0.29	2.39
Average				56	203	24.4	3.7	0.45	0.27	2.25
Casein.	9.3	2051♂	65	19	169	15.7	8.9	0.83	0.11	1.21
		2595♂	65	29	184	17.1	6.4	0.59	0.16	1.69
		2603♂	64	27	199	18.5	7.4	0.68	0.14	1.46
Average				25	184	17.1	7.6	0.70	0.14	1.45

than were used for these experiments in order properly to minimize errors caused by such individual variations.

Table I shows that when the food contained 7.9 per cent of lactalbumin the maximum gain of body weight per gm. of protein eaten was 3.0 gm., which probably represents approximately the

maximum growth-promoting capacity of lactalbumin when fed to albino rats under the conditions of these experiments.

Table II gives similar data obtained with casein as the sole protein of the diet. Here the greatest gain per gm. of protein eaten was 2.25 gm., the food containing 12 per cent of casein. To make this maximum gain the rats ate on the average 24.4 gm. of casein and 203 gm. of food and gained 56 gm. When the food contained 7.9 per cent of lactalbumin the rats ate on the average 19.7 gm. of lactalbumin and 252 gm. of food and gained 59 gm. Thus to make the same gain in the same time under the conditions of maximum efficiency 24 per cent more protein and 20 per cent more food were needed when the protein was casein than when it was lactalbumin.

When an animal is restricted to such a quantity of protein that a maximum gain of body weight is made per unit of protein eaten, it grows at less than the normal rate. A longer time therefore is required to make a given gain of weight, and consequently more food is needed for maintenance than if growth had not thus been delayed. In these experiments nearly the same amount of food was eaten whether this contained 6.2 or 16.2 per cent of lactalbumin (see Table I), but the gain in weight was almost twice as great on the high as on the low protein food; namely, 61 and 35 gm. respectively, or 0.32 and 0.17 gm. of gain per gm. of *food* eaten. Although growth on the 6.2 per cent ration was made with the least expenditure of *protein*, the consumption of *food* was almost twice as great per gm. of gain as that on the 16.2 per cent diet. Consequently, although an economy in the consumption of protein may be effected by reducing its concentration in the diet, this is necessarily accompanied by a larger consumption of food.

The data given in Tables I and II may be compared with those previously obtained in attempting to establish by other methods the relative value for growth of lactalbumin and casein.[1] Series A of the earlier experiments differed from those described in the present paper in that the food intake was restricted by supplying the diet in weighed quantities, estimated to be about 10 per cent less than was needed for full normal growth, and also in being continued for 11 weeks. From the data given in Table II of the earlier paper we have calculated the gains in body weight

per gm. of *protein* eaten for the entire 11 weeks, and also, for comparison with the experiments just described, for periods of 4 weeks following the time at which these rats had reached a body weight of about 70 gm. In recalculating these results we have estimated the protein from the nitrogen content of the diet whereby the nitrogen of the "protein-free milk" was included, as was done for the experiments described in this paper.

		Gain per gm. of food or protein eaten in			
		11 wks.		4 wks.	
		Food.	Protein.	Food.	Protein.
	per cent	*gm.*	*gm.*	*gm.*	*gm.*
Lactalbumin.	16.0	0.279	1.74	0.325	2.03
	11.1	0.228	2.06	0.240	2.16
	9.2	0.176	1.91	0.200	2.17
Casein.	17.4	0.240	1.38	0.273	1.57
	12.0	0.194	1.62	0.193	1.61
	9.2	0.162	1.76	0.164	1.77

The gains of weight per gm. of *protein* eaten during the 4 week periods were slightly higher than during the 11 week periods, presumably because the former coincide with the time of most rapid growth. The maximum gains were decidedly less in these 4 week periods than those obtained in our new experiments, probably because in the earlier trials growth also was

CORRECTION.

On page 226, Vol. XXXVII, No. 2, February, 1919, line 12, for *20 per cent more food*, read *20 per cent less food*.

proteins for growth. The comparison just made gives additional reasons for rejecting such methods of experimentation.

In Chart II of our earlier paper we showed that rats made equal gains of weight in equal periods of time and ate practically

maximum growth-promoting capacity of lactalbumin when fed to albino rats under the conditions of these experiments.

Table II gives similar data obtained with casein as the sole protein of the diet. Here the greatest gain per gm. of protein eaten was 2.25 gm., the food containing 12 per cent of casein. To make this maximum gain the rats ate on the average 24.4 gm. of casein and 203 gm. of food and gained 56 gm. When the food contained 7.9 per cent of lactalbumin the rats ate on the average 19.7 gm. of lactalbumin and 252 gm. of food and gained 59 gm. Thus to make the same gain in the same time under the conditions of maximum efficiency 24 per cent more protein and 20 per cent more food were needed when the protein was casein than when it was lactalbumin.

When an animal is restricted to such a quantity of protein that a maximum gain of body weight is made per unit of protein eaten, it grows at less than the normal rate. A longer time therefore is required to make a given gain of weight, and consequently more food is needed for maintenance than if growth had not thus been delayed. In these experiments nearly the same amount of food was eaten whether this contained 6.2 or 16.2 per cent of lactalbumin (see Table I), but the gain in weight was almost twice as great on the high as on the low protein food; namely, 61 and 35 gm. respectively, or 0.32 and 0.17 gm. of gain per gm. of *fo*
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per gm. of *protein* eaten for the entire 11 weeks, and also, for comparison with the experiments just described, for periods of 4 weeks following the time at which these rats had reached a body weight of about 70 gm. In recalculating these results we have estimated the protein from the nitrogen content of the diet whereby the nitrogen of the "protein-free milk" was included, as was done for the experiments described in this paper.

		Gain per gm. of food or protein eaten in			
		11 wks.		4 wks.	
		Food.	Protein.	Food.	Protein.
	per cent	*gm.*	*gm.*	*gm.*	*gm.*
Lactalbumin.	16.0	0.279	1.74	0.325	2.03
	11.1	0.228	2.06	0.240	2.16
	9.2	0.176	1.91	0.200	2.17
Casein.	17.4	0.240	1.38	0.273	1.57
	12.0	0.194	1.62	0.193	1.61
	9.2	0.162	1.76	0.164	1.77

The gains of weight per gm. of *protein* eaten during the 4 week periods were slightly higher than during the 11 week periods, presumably because the former coincide with the time of most rapid growth. The maximum gains were decidedly less in these 4 week periods than those obtained in our new experiments, probably because in the earlier trials growth also was restricted by the amount of *food* supplied, and not solely by the protein eaten. When growth is limited by food intake protein can be used as a source of energy and consequently a smaller part may be available for growth than when sufficient energy is supplied in other forms and growth is determined solely by protein.

In our former paper we called attention to criticisms, based on other grounds, which might be made to Series A of the earlier experiments, which was designed to show the relative value of proteins for growth. The comparison just made gives additional reasons for rejecting such methods of experimentation.

In Chart II of our earlier paper we showed that rats made equal gains of weight in equal periods of time and ate practically

the same amount of food when diets of essentially the same composition were fed, but containing 8 per cent of lactalbumin, 12 per cent of casein, or 15 per cent of edestin. While the method employed for these experiments shows the *relative* growth-promoting power of these proteins it does not show their maximum efficiency. Thus the gain of weight per gm. of protein eaten during the 8 weeks of these experiments was for lactalbumin 2.34 gm., for casein 1.70, and for edestin 1.35 gm. These figures for lactalbumin and casein are decidedly lower than the maxima found by the method described in this paper, and the difference in the relative value of these proteins appears greater than it actually is.

For the investigator we believe that this new method, which shows with some degree of accuracy the maximum efficiency of individual proteins, or mixtures of them, for growth, will be of use. By its aid we can determine and express numerically the efficiency of combinations of proteins and compare this with that of either one alone. Differences in food intake and rate of growth are largely eliminated and experimental data can be used which cannot be compared in any other way.

This method has its limitations, for obviously when the protein of the diet is capable of promoting growth only at a very slow rate the amount of protein eaten per gm. of gain made will approach infinity as the gain approaches zero. The error thus introduced of course affects all comparisons made between figures obtained by the simple method of dividing the gain of weight by the gm. of protein eaten, but when the rate of growth on the two proteins thus compared is fairly rapid the magnitude of the error is small.

The practical feeder does not wish to know what quantity of a given protein is the smallest which he can use to secure a given amount of gain, if this quantity can only be used under unprofitable conditions. Rather does he want to know the least proportion which will give him the greatest gain in the shortest time, for although he may thereby waste some protein he may save food. The method described in our earlier paper (Series B) is better adapted to the use of the dietitian or the agriculturist in determining the relative value of proteins for growth than the method now described in this paper.

The effect of *restricted food* intake on gains made per unit of *food* eaten is shown by the following figures:

Gain per Gm. of Food Eaten.

	Protein fed.	Restricted intake.	Protein fed.	Unrestricted intake.
	per cent	*gm.*	*per cent*	*gm.*
Lactalbumin.	16.0	0.33	16.2	0.32
	11.1	0.24	10.3	0.27
	9.2	0.20	7.9	0.24
Casein.	21.0	0.23		
	17.4	0.24	17.4	0.30
	12.0	0.19	11.0	0.27
	9.2	0.16	9.3	0.14
Edestin.	17.9	0.27	17.9	0.25
	12.4	0.16	12.4	0.20
	9.2	0.10	9.6	0.13

Comparing the gains made on diets containing similar percentages of each protein when the food intake was restricted, with those made when it was unrestricted, it is seen that with only three exceptions these were greater under the latter conditions of feeding. Economy of food can be effected only by supplying the young animal with as much as it will eat; economy of protein only by reducing the nutritive ratio below that at which the normal rate of growth can be maintained.

Economy in nutrition during growth depends upon a correct adjustment between the proportion of protein and the total energy supplied: furthermore, *the optimum of protein is determined not only by the absolute amount furnished, but also by its quality.*

NEW TITRATION METHOD FOR THE DETERMINATION OF URIC ACID IN URINE.*

By J. LUCIEN MORRIS.

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(Received for publication, November 26, 1918.)

Determinations of pure uric acid have been made by permanganate titrations for many years. The presence of other substances with uric acid in body fluids, which give permanganate values, has necessitated the separation of uric acid before the titration could be carried out. Such separations have never been perfect. Whether thrown down as such by treatment with stronger acids or precipitated as urates, there has always been a part of the uric acid left in solution. Hopkins[1] came nearest the desired separation with his method of precipitation of ammonium urate. Probably the modification of Folin and Shaffer[2] has proved most useful of all, but it is distinctly imperfect since precipitation is incomplete even after 48 hours.

The quantitative precipitation of uric acid by adding a soluble zinc salt and then making the solution alkaline with sodium carbonate has been described in an earlier paper.[3] At that time it was mentioned that Ganassini[4] had described a qualitative test for uric acid based upon a similar precipitation. It later was

* Preliminary reports of this work were presented before the 1916 and 1917 annual meetings of the Society of Biological Chemists. Cf. Morris, J. L., *J. Biol. Chem.*, 1917, xxix, p. xiii; 1918, xxxiii, p. xxi.

[1] Hopkins, F. G., *Guy's Hosp. Rep.*, 1891, xlviii, 299.

[2] Folin, O., and Shaffer, P. A., *Z. physiol. Chem.*, 1901, xxxii, 552.

[3] Morris, *J. Biol. Chem.*, 1916, xxv, 205.

[4] Ganassini, D., *Boll. Soc. med. chir.*, 1908, No. 1; abstracted in *Biochem. Centr.*, 1909, viii, 250.

discovered that Bellocq[5] knew of this reaction in 1900 and used it for determining uric acid. His method involved dissolving the precipitate in hydrochloric acid and then weighing that portion of the uric acid precipitated by hydrochloric acid. It is easy to demonstrate that all the uric acid is not precipitated by such treatment. Kashiwabara[6] described a similar procedure for separating uric acid, then removed the zinc as sulfide before the final precipitation with hydrochloric acid. Both these methods are long and can give, at best, only approximate results. The fact, demonstrated by the writer,[3] that uric acid can be completely and instantly precipitated as the zinc compound seemed to warrant efforts to combine this means of separation with a satisfactory method of determination. A combination with the colorimetric procedure of Folin and Denis[7] was attempted but many difficulties were encountered. Among these the most serious was fading due to the presence of the zinc ion. The long used permanganate titration was then applied with more promising results.

A form of analysis which resulted from the combination of zinc precipitation and permanganate titration has been described before.[8] Two preliminary precipitations for the removal of interfering substances were required before the uric acid could be separated and, though the end-point of the permanganate titration in acetic acid solution was recognizable within a few per cent, it was not entirely satisfactory. An attempt was made to improve the method by using an iodine end-point. The oxidation was carried out by using an excess of permanganate and this was followed with a larger excess of potassium iodide. It was then possible to titrate with standard thiosulfate. This method made use of the more dependable starch iodide end-point but at the cost of more time-consuming work and did not entirely eliminate the error due to spontaneous reduction of permanganate. The double titration was modified in many ways and finally was combined into one titration in which the oxidation takes place in a solution alkaline with sodium bicarbonate.[8]

[5] Bellocq, A., *J. pharm. et chim.*, 1900, xii, 103.
[6] Kashiwabara, M., *Z. physiol. Chem.*, 1913, lxxxiv, 223.
[7] Folin, O., and Denis, W., *J. Biol. Chem.*, 1912–13, xiii, 469.
[8] Morris, *J. Biol. Chem.*, 1917, xxix, p. xiii; 1918, xxxiii, p. xxi.

The use of bicarbonate was valuable for it controlled the hydrogen ion concentration so well that a mild selective oxidation took place by which all the uric acid was decomposed before any iodine was set free from the iodide. The blue starch iodide end-point could thus be used directly in the permanganate titrations. On the same principle it seemed that probably many of the substances which interfere with permanganate titrations in acid solution would be less easily oxidized than iodides and thus not interfere with the titration in sodium bicarbonate. The following substances were added to test titrations without any apparent effect: allantoin, caffeine, dextrose, hippuric acid. Phenols affected the end-point, causing a slow fading of the blue color, but earlier work[3] had shown that the zinc precipitation separated uric acid quantitatively from phenols. The results from the few substances tested suggested that possibly the preliminary precipitations of interfering substances were not necessary when oxidizing in the presence of sodium bicarbonate, and investigation showed that such was substantially the case. This made it possible again to shorten the method. The final procedure, described in this paper, requires 30 to 40 minutes for the complete determination. It has proved entirely satisfactory for urine. For the much smaller quantities of uric acid in blood it gives good results also but its usefulness is limited, as is that of all other methods, by the imperfect procedures for removing proteins without at the same time carrying down other substances. When applied to the filtrates from the usual 0.01 N acetic acid precipitation the results agree with those obtained with the colorimetric method.

The steps by which the writer developed the volumetric method here described are given in detail, not only because of his belief that some light has been thrown upon the oxidation of uric acid under varying conditions but in order also to show that the idea of a titration method for determining uric acid has been consistently followed by him for more than 2 years. Curtman and Lehrman[9] have published a volumetric method for uric acid in which the underlying principles are substantially the same as those previously set forth by the writer.[8]

[9] Curtman, L. J., and Lehrman, A., *J. Biol. Chem.*, 1918, xxxvi, 157.

Method.

Reagents.

Preparation of Standard Potassium Permanganate.—A stock 0.1 N solution is made up in the usual manner (3.166 gm. per liter) and kept in a tightly stoppered amber colored bottle. After the usual preliminary period, during which the value decreases somewhat, the value remains as nearly constant as its use in the preparation of 0.002 N solutions requires. When the dilute solution is needed, 10 cc. of the stock solution are measured with a pipette into a 500 cc. volumetric flask and made up to the mark. The resulting 0.002 N solution may then be standardized against a standard uric acid-phosphate solution[10] by a titration such as that described later in this paper for the uric acid separated from urine. Three different 0.1 N stock solutions have upon dilution given values of 0.140, 0.143, and 0.139 mg. per 1 cc. of the 0.002 N solutions. If it is considered more convenient, the stock permanganate solution may be standardized in one of the usual ways and its equivalent value in uric acid calculated on the basis of 1 cc. of 0.1 N permanganate being equal to 7.50 mg.[1] or 7.22 mg.[11] of uric acid. According to our experience with the titration in bicarbonate solution 1 cc. of 0.1 N permanganate is equivalent to 7.32 mg. of uric acid. The dilute permanganate solution keeps its value for a relatively short time, but in no case have we found it to vary appreciably during a day's work.

Other Reagents Required.—Saturated disodium phosphate solution; 10 per cent zinc chloride solution; 10 per cent calcium chloride solution; 10 per cent potassium iodide solution; 20 per cent sodium carbonate solution; 0.5 per cent soluble starch solution (starch will serve but soluble starch gives a better endpoint); solid sodium bicarbonate.

Determination.

The procedure as now used is as follows: Pipette 5 cc. of urine into a 50 cc. or 100 cc. centrifuge tube and add 3 cc. of 10 per

[10] Benedict, S. R., and Hitchcock, E. H., *J. Biol. Chem.*, 1915, xx, 619.
[11] von Ritter, G., *Z. physiol. Chem.* 1895–96, xxi, 290.

cent zinc chloride. Add 25 cc. of water, mix with a stirring rod, put in a small piece of litmus paper, and add enough 20 per cent sodium carbonate, with stirring, to turn the litmus entirely blue (not the violet shade of neutrality or weak alkalinity). A heavy precipitate of zinc carbonate, zinc phosphate, and zinc urate separates from a clear liquid. Now centrifugalize for 2 or 3 minutes; carefully pour off the liquid, discarding it. The precipitate is sufficiently packed down to remain in the tube. Dissolve this in dilute hydrochloric acid, with stirring. To the acid solution add 15 cc. of saturated disodium phosphate and, if necessary, a few drops more of dilute hydrochloric acid to dissolve a precipitate of zinc phosphate. Finally pour in 2 cc. of 3 per cent uranium acetate and, while stirring, again make the contents of the tube alkaline with sodium carbonate. Centrifugalize for 2 or 3 minutes and pour clear liquid carefully into a 300 to 500 cc. Erlenmeyer flask for titration. In the presence of the large excess of phosphates none of the uric acid will precipitate, while the zinc and uranium have carried down all other substances which would have given a value in the permanganate titration.

Acidify the alkaline liquid containing the uric acid with hydrochloric acid and add 25 cc. of 10 per cent calcium chloride. Then add excess of solid sodium bicarbonate. Calcium phosphate will precipitate when the acid reaction has been neutralized. An excess of sodium bicarbonate beyond this point is desirable. To the very weakly alkaline solution so prepared for titration add 1 cc. of 10 per cent potassium iodide, 3 cc. of 0.5 per cent soluble starch, and enough distilled water to make the entire volume within a few cc. of 250 cc. Now titrate with 0.002 N potassium permanganate until a blue shade spreads throughout the liquid. The color of the drop of permanganate as it hits the surface changes from pink to blue and assists in judging the approach of the end-point. Comparison of the flask from time to time with another flask containing the duplicate awaiting titration or a blank arranged for the purpose has been found to assist in arriving at the end-point. The number of cc. of standard permanganate run in from the burette needs a correction for a blank value corresponding to the amount of free iodine necessary in 250 cc. to show a perceptible blue color. This can easily be

determined for the standard being used by titrating a blank containing 1 cc. of 10 per cent potassium iodide, 3 cc. of 0.5 per cent starch, and 250 cc. of water. It has had a value of 0.8 cc. of permanganate in our experience. Subtracting this figure from the observed reading gives the actual number of cc. of 0.002 N permanganate required for the uric acid present. Multiplying this by the value of each cc. of the permanganate gives the amount of uric acid present in 5 cc. of urine.

TABLE I.

Comparative Uric Acid Estimations.

Urine.	Benedict modification of Folin-Denis colorimetric method.	Proposed zinc precipitation-alkaline oxidation method.	Folin-Shaffer ammonium urate-acid oxidation method.
	gm. per 24 hrs.	*gm. per 24 hrs.*	*gm. per 24 hrs.*
F. H.*	0.592	0.592	0.527
	0.586	0.592	
M. S.*	0.505	0.495	0.464
	0.513	0.507	
Dr. C.†	0.597	0.573	
	0.577	0.577	
Mrs. C.‡	0.270	0.318	0.231
Mrs. X.‖	0.323	0.357	0.279

* Normal.
† Nephritis.
‡ Toxic vomiting of pregnancy.
‖ Pregnancy.

DISCUSSION.

It will be noted from the typical results recorded in Table I that the agreement between duplicates of the zinc precipitation-alkaline oxidation procedure is as good as those with the colorimetric method. In normal urines the values with both are practically identical. There are some irregularities in the figures given for the pathological cases. These irregularities are present in most of the pathological urines so far examined. Attempts have been made to locate the cause for this in the proposed method by the addition of such substances as might possibly be

present. The absence of any effect which can be attributed to any of the substances tried (Table II) leaves the difficulty unsolved. The figures given for the usual volumetric method (ammonium urate precipitation-acid oxidation) show that all results with that method are decidedly low.

Table II lists a few substances that might be present in urine and might, because of their nature, be suspected of affecting the titration values. The variation in titration values, with the exception of phenols, is no more in their presence than is found between separate titrations of pure solutions. Phenol gives a value in the titration and the end-point is a fading one, indicating the continued oxidation of phenol. This substance, of all those

TABLE II.

Selective Oxidation of Uric Acid by Potassium Permanganate in Solution Made Alkaline with Sodium Bicarbonate.

Uric acid standard (5 cc. = 1 mg.).	Amount of 0.002 N $KMnO_4$ required for uric acid.	Substance added.	Amount of 0.002 N $KMnO_4$ required for substance + uric acid.
cc.	*cc.*		*cc.*
5	7.0	Caffeine.	7.1
5	7.1	Allantoin.	7.0
5	7.2	Hippuric acid.	7.0
5	7.1	Dextrose.	7.2
5	7.0	Phenol.	7.8+*

* End-point indefinite and fading.

tried, most closely resembles uric acid in the selective oxidation process used. Fortunately the separation of uric acid from phenols by precipitation of the former as the zinc salt[3] precludes the presence of phenols in the final titration.

SUMMARY.

A volumetric method for the determination of small amounts of uric acid has been developed and described in detail for urine. It is based upon the instantaneous and complete precipitation of uric acid as the zinc salt and a single direct titration with permanganate in a solution alkaline with sodium bicarbonate. The end-point used is the blue starch iodide color and, because of the

mild nature of the oxidation and the excess iodide present, there is no fading to confuse the end-point.

The results on the usual blood filtrate obtained by precipitating proteins with dilute acetic acid agree with those obtained with the colorimetric method. Because of the usual difficulties which attend this preliminary precipitation of blood proteins, no detailed report has been made here of results of this application of the method. Attempts are still being made to find a precipitant of proteins which will make it possible to obtain from blood entirely reliable results with this sensitive volumetric method.

COMPARATIVE DISTRIBUTION OF UREA, CREATININE, URIC ACID, AND SUGAR IN THE BLOOD AND SPINAL FLUID.*

By VICTOR C. MYERS and MORRIS S. FINE.

(From the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital, New York.)

(Received for publication, December 30, 1918.)

In previous papers,[1] it has been shown that as the permeability of the kidney is lowered in conditions of renal insufficiency this becomes evident in the blood, first by a retention of uric acid, later by that of urea, and lastly by that of creatinine, indicating that creatinine is the most readily eliminated of these three nitrogenous waste products, and uric acid the most difficultly eliminated, with urea standing in an intermediate position. Judging from the comparative composition of the blood and urine, the kidney normally concentrates the creatinine 100 times, the urea 80 times, but the uric acid only 20 times. There is apparently little difference in the ability of the kidney to excrete creatinine and urea, although there is a marked difference between these two substances and uric acid.

In connection with these observations, it appeared of interest to us to compare the concentration of these waste products in the blood with those of the spinal fluid in cases with normal kidney activity and with nitrogen retention. With the rise in the concentration of these substances in the blood as the result of renal impairment, there must be a corresponding change in the physical factors influencing their content in the spinal fluid. Are the well known physical properties of these substances, such as solubility and diffusibility, chiefly concerned in determining

* Reported in *Proc. Soc. Exp. Biol. and Med.*, 1915–16, xiii, 126.

[1] Myers, V. C., and Fine, M. S., *Arch. Int. Med.*, 1916, xvii, 570; Chace, A. F., and Myers, V. C., *J. Am. Med. Assn.*, 1916, lxvii, 929. Watanabe, C. K., *Am. J. Med. Sc.*, 1917, clix, 76.

how well the concentration of these substances in the spinal fluid will keep pace with the blood, or are other factors concerned?

The recent studies of Cushing and his coworkers[2] have given us more definite information regarding the secretion of the spinal fluid. It appears to be secreted chiefly by the choroid plexus, the cells of which are relatively impermeable to the passage from the blood stream of drugs and such substances as bile pigments. An observation made by one of us[3] several years ago is of interest in this connection. Immediately after death, as soon as ½ hour, the content of potassium salts in the spinal fluid rises to five times the normal—to the approximate concentration of the blood plasma.

It is now well known that the various membranes of the body are very permeable to urea, resulting in an even distribution[4] of this waste product throughout the tissues of the body, and Cullen and Ellis[5] have strikingly pointed this out in the case of the blood and spinal fluid, an observation also made by Woods[6] at about the same time. We have likewise recorded a few observations on the spinal fluid in connection with analyses of the blood.[7] Data on the spinal fluid of children have been reported by Leopold and Bernhard.[8] In their normal cases they found an average urea nitrogen content of 9.9 mg. to 100 cc., 0.9 mg. of creatinine, and no uric acid, although in some of their pathological cases traces of uric acid were found. In edema fluids it has been pointed out by Denis,[9] and also by Bernhard,[10] that the concentration of the non-protein nitrogenous constituents is comparable with that of the blood.

Our own observations recorded in Table I were made during 1915 and the early part of 1916, and include estimations of the

[2] Cushing, H., *J. Med. Research*, 1914–15, xxxi, 1. Weed, L. H., *ibid.*, 21, 51, 93. Wegefarth, P., *ibid.*, 119, 149. Wegefarth and Weed, *ibid.*, 167.

[3] Myers, V. C., *J. Biol. Chem.*, 1909, vi, 115.

[4] Marshall, E. K., Jr., and Davis, D. M., *J. Biol. Chem.*, 1914, xviii, 53.

[5] Cullen, G. E., and Ellis, A. W. M., *J. Biol. Chem.*, 1915, xx, 511.

[6] Woods, A. C., *Arch. Int. Med.*, 1915, xvi, 577.

[7] Myers, V. C., and Fine, M. S., *J. Biol. Chem.*, 1915, xx, 391.

[8] Leopold, J. S., and Bernhard, A., *Am. J. Dis. Child.*, 1917, xiii, 34.

[9] Denis, W., *Arch. Int. Med.*, 1917, xx, 879.

[10] Bernhard, A., *Interstate Med. J.*, 1918, xxv, 188.

urea, creatinine, creatine, uric acid, and sugar of both the blood and spinal fluid. The estimations of the urea, creatinine, and uric acid were carried out as already described,[11] while the sugar was determined by a modification of the Lewis and Benedict method.[12] Creatine was determined by the method of Folin.[13] Since these studies were completed it has been shown by several workers that the results with this method are too high. The figures for creatine have, however, been retained in our table as a matter of record. The blood and spinal fluids were obtained simultaneously, except as otherwise noted in Table I.

DISCUSSION.

Table I presents observations on blood and spinal fluid in fifteen cases. These patients were suffering from nephritis of various stages of severity, and gave chemical blood pictures varying from practically normal to excessive retention of uremia. The data are arranged according to the magnitude of the blood urea. When the blood and spinal fluid were obtained simultaneously, there was a slight lag in the urea of the spinal fluid in comparison with the blood. Averaging the whole series, the content of the spinal fluid urea amounts to 88 per cent of that of the blood. The first seven cases of the series show high figures for the creatinine of the blood, and, with one exception, the figures for the spinal fluid are likewise increased, though not in proportion to the increase found in the case of the urea. Case 5, in which the creatinine of the spinal fluid was not notably changed, was an acute and rapidly fatal case. The spinal fluid was taken 1 day previous to the blood. For the series, the creatinine of the spinal fluid represents 46 per cent of that of the blood. Apparently not more than a trace of uric acid is normally present in the spinal fluid. When sufficient fluid was available for a satisfactory estimation in these pathological cases, it was possible to obtain enough uric acid in some instances to give a fairly satisfactory color reaction. The largest amounts of uric acid were obtained in the first seven subjects, as in the case of the creati-

[11] Myers, V. C., and Fine, M. S., *Arch. Int. Med.*, 1916, xvii, 570.
[12] Myers, V. C., and Bailey, C. V., *J. Biol. Chem.*, 1916, xxiv, 147.
[13] Folin, O., *J. Biol. Chem.*, 1914, xvii, 475.

TABLE I.

Comparative Distribution of Urea, Creatinine, Creatine, Uric Acid, and Sugar in Blood and Spinal Fluid.

Case No.	Age.	Sex.	Urea.		Creatinine.		Creatine.		Uric acid.		Sugar.		Remarks.
			Blood.	Spinal fluid.	Blood.	Spinal fluid.	Blood.	Spinal fluid.	Blood.	Spinal fluid.	Blood.	Spinal fluid.	
			Mg. per 100 cc.								*per cent*	*per cent*	
1	17	♀	288	306	14.5	4.8	11.5	1.0	12.5	0	0.19	0.09	Interstitial nephritis, uremia; died. Spinal fluid obtained 1 day previous to blood.
2	34	♂	208	143	11.0	4.2	5.6	0.8	9.1	0.7	0.14	—	Interstitial nephritis, uremia; died. Spinal fluid obtained 4 days after blood.
3	57	♂	171	—	4.8	3.9	8.9	0.5	8.0	1.5	0.14	—	Interstitial nephritis; improved.
4	3	♂	150	124	4.8	2.1	2.9	1.3	11.8	0.9	0.14	0.12	Scarlatinal nephritis.
5	22	♂	139	113	4.5	1.5	7.2	1.6	7.3	Trace.	0.13	0.07	Syphilis; spinal fluid taken 1 day before blood.
6	30	♂	103	105	10.8	4.0	3.6	3.1	4.7	0.5	0.16	0.09	Interstitial nephritis.
7	46	♂	103	96	4.8	2.1	6.4	1.3	8.6	0.9	0.27	0.12	Interstitial nephritis, spinal fluid taken 1 day after blood.
8	55	♂	96	96	2.3	1.8	8.6	1.0	5.7	0.1	0.19	0.09	Cheyne-Stokes respiration.
9	48	♂	90	83	2.0	1.3	4.2	1.2	5.6	0	0.15	0.11	Chronic diffuse nephritis.
10	4½	♂	83	77	3.1	1.3	4.1	1.0	5.6	0.05	0.15	0.08	Scarlatinal nephritis.

TABLE I—*Concluded.*

Case No.	Age.	Sex.	Urea.		Creatinine.		Creatine.		Uric acid.		Sugar.		Remarks.
			Blood.	Spinal fluid.	Blood.	Spinal fluid.	Blood.	Spinal fluid.	Blood.	Spinal fluid.	Blood.	Spinal fluid.	
			Mg. per 100 cc.										
											per cent	*per cent*	
11	25	♀	43	41	2.6	1.4	3.7	1.2	6.4	Trace	0.14	0.08	Chronic diffuse nephritis.
12	50	♂	39	34	2.8	1.3	3.3	1.4	3.9	0	0.14	0.08	Syphilis.
13	28	♂	32	39	2.4	1.8	—	0.9	6.3	−0.5	—	0.09	" Spinal fluid taken 11 days after blood.
14	46	♀	26	24	2.5	1.2	2.9	1.2	3.0	−0.05	0.14	—	Gastritis.
15	71	♂	26	24	2.5	1.8	6.6	1.4	3.8	0.3	0.12	0.08	Chronic interstitial nephritis.
Average...			107	94	5.0	2.3	5.6	1.3	6.8	0.4	0.16	0.09	

nine, although there does not appear to be any close relationship between the varying amount in the blood and spinal fluid. Uric acid does not readily pass to the spinal fluid, since the amount present in this fluid represents only about 5 per cent of that found in the blood.

The comparative distribution of the sugar in the blood and spinal fluid is of interest, since the mechanism by which sugar is eliminated by the kidney is apparently quite different from that by which urea, creatinine, and uric acid are excreted. It is now generally recognized that sugar is present in the spinal fluid in amounts somewhat below that of the blood, a fact borne out by the present series of observations. Such slight differences as are present in the sugar of the spinal fluid reflect differences in the blood sugar. An observation on a case of pronounced diabetes is of interest in this connection. With a blood sugar of 0.438 per cent, the spinal fluid contained 0.30 per cent sugar. For the series, the sugar concentration of the spinal fluid amounted to about 60 per cent of the quantity present in the blood.

If the differences in concentration noted above may be regarded as representing the relative permeability of the cells separating the blood and spinal fluid, one notes that the extent of passage from the blood into the spinal fluid is greatest for urea, less for sugar, still less for creatinine, and least for uric acid. This also represents the order of their solubility in water, and apparently the order of their diffusibility through the various tissues and membranes, observations in agreement with the view of McClendon[14] that the spinal fluid is an ultrafiltrate. As regards the kidney, the findings harmonize with the fact that uric acid is excreted with difficulty, but do not explain why creatinine is apparently more readily eliminated than urea. The solubility of urea is about ten times that of creatinine and its diffusibility considerably greater, although it may be noted that normally there is about fifteen times as much urea eliminated. The greater amount of work necessary for the kidney to do in the case of urea would seem to afford the only physical explanation as to why creatinine is the more readily eliminated of these two waste products.

SUMMARY.

Comparative analyses of blood and spinal fluid are reported on fifteen cases, showing varying degrees of nitrogen retention. The concentration of urea in the spinal fluid averaged 88 per cent of that of the blood, the concentration of creatinine 46 per cent, that of uric acid 5 per cent, and that of sugar 57 per cent. These percentage differences represent the order of their solubility in water, and apparently also the order of their diffusibility.

[14] McClendon, J. F., *J. Am. Med. Assn*, 1918, lxx, 977.

CREATINURIA AND ACIDOSIS.

By W. DENIS and A. S. MINOT.

(From the Chemical Laboratory of the Massachusetts General Hospital, Boston.)

(Received for publication, December 28, 1918.)

The results of a series of experiments recently carried on in this laboratory[1] seem to prove the close connection between protein intake and the production of creatinuria in man. Briefly summarized our results indicated that in children, in two normal women, and in persons suffering from hyperthyroidism, the amount of creatine present in the urine could be increased, or decreased, at will by changes in the protein content of the diet.

It has been shown by Underhill[2] that in rabbits creatine excretion can be produced by the administration of a diet high in acid salts (oats and corn), and decreased to the vanishing point, either by the administration of food of an alkaline nature such as carrots, or by the use of a ration composed of an appropriate mixture of the above acid and basic foods; if, however, to this mixed ration hydrochloric acid is added, creatine excretion promptly begins, and continues as long as the administration of acid is kept up. On the basis of this experimental work Underhill has suggested the possibility of a relation between acidosis and creatine excretion.

While in our opinion the results of our feeding experiments summarized above may be considered as evidence in favor of the protein theory of creatine origin, it must be admitted that the high protein diets used by us were of a highly acid character, while our low protein diets consisted largely of alkaline vegetarian foods. It has therefore seemed undesirable to leave the subject

[1] Denis, W., *J. Biol. Chem.*, 1917, xxix, 447. Denis, W., and Kramer, J. G., *ibid.*, 1917, xxx, 189. Denis, W., and Minot, A. S., *ibid.*, 1917, xxxi, 561.

[2] Underhill, F. P., *J. Biol. Chem.*, 1916, xxvii, 127.

without carrying out on human subjects a few experiments along the same general lines used by Underhill in his work on rabbits.

Preliminary experiments soon showed that it was impossible to administer hydrochloric acid to human subjects in amounts sufficiently large to make our work in any way comparable to that of Underhill; we have therefore been compelled to adopt the reverse procedure, *i.e.* to feed a highly acid diet, and after creatine excretion was established to administer sodium bicarbonate in amounts sufficient to keep the urine alkaline to litmus.[3]

Our experimental subjects consisted of two normal boys, four women suffering from hyperthyroidism, and two normal women.

The boys and the four hyperthyroid cases were patients of this hospital; the two former were surgical cases who were being treated for fractures of the leg; they were both in good physical condition, had been subjected to no surgical procedures for 2 weeks before becoming experimental subjects, and may be considered as essentially normal.

In Experiments I to VI the subjects were given the following foods: bread, butter, eggs, milk, cheese, gelatin, one orange, and one apple. After a day or two of preliminary feeding in which the capacity of each subject was determined, a diet list composed of the above foods was made out to which the patients were required to adhere during the experimental period.

The sodium bicarbonate was administered in four equal doses at 3 hour intervals between 7 a.m. and 7 p.m.

Observations on body temperature were made at 12 hour intervals, but as no abnormal figures are recorded these data are not included in the tables.

Creatinine and creatine determinations were made by Folin's micro methods using purified picric acid; the determinations of

[3] Recently Steenbock and Gross (*J. Biol. Chem.*, 1918, xxxvi, 265) have carried on a series of experiments on pigs and have shown that with these animals "protein feeding, if sufficiently intensive, will always produce creatinuria or if already present will increase it in degree. This effect obtains even during alkalosis and cannot be attributed to acids resulting from the metabolism of the protein molecule." They have further shown that administration of amounts of hydrochloric acid equivalent to the potential acidity of the ingested protein are entirely ineffective in the production of creatinuria.

hydrogen ion concentrations were made by the colorimetric technique of Henderson and Palmer.

On the whole the experimental results presented appear to demonstrate no definite connection between changes in acid-base equilibrium and creatine excretion. In Experiments I and II (normal boys), III (hyperthyroidism), and VII (normal woman) no definite change in creatine excretion was noted. In Subject 5 (hyperthyroidism) a distinct increase occurred, while in Subjects 4 and 6 (hyperthyroidism) and Subject 8 a decrease occurred.

EXPERIMENT I.

Subject 1. Male, 12 Years, Weight 31 Kilos.

Date.	24 hour volume.	Creatinine.	Creatine.	Total nitrogen.	Sodium bicarbonate taken.
1918	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*
Feb. 23–24	500	0.58	0.22	6.62	0
" 24–25	400	0.58	0.21	6.25	0
" 25–26	780	0.56	0.20	9.36	10
" 26–27	530	0.58	0.17	10.60	10
" 27–28	1,660	0.51	0.17	10.29	10
" 28–Mar. 1	770	0.46	0.25	9.39	10
Mar. 1– 2	900	0.52	0.24	10.26	0
" 2– 3	620	0.42	0.22	7.44	0
" 3– 4	960	0.52	0.18	7.39	0
" 4– 5	—	—	—	—	0
" 5– 6	1,020	0.54	0.22	9.18	10
" 6– 7	950	0.52	0.14	9.26	10
" 7– 8	980	0.56	0.15	11.22	10
" 8– 9	1,180	0.54	0.20	10.20	10
" 9–10	640	0.53	0.23	8.00	0
" 10–11	840	0.55	0.16	9.32	0
" 11–12	800	0.50	0.29	9.60	0

While the experiments outlined above were in progress several papers have appeared bearing on the problem of the relation of protein intake to creatine excretion.

Rose[4] has published an account of experiments extending over periods of several weeks, the results of which lead her to conclude that "the creatine output in women is very irregular; . . . is not definitely influenced by the amount of protein in the diet." This

[4] Rose, M. S., *J. Biol. Chem.*, 1917, xxxii, 1.

series of experiments was apparently planned to furnish evidence concerning the influence of the sexual cycle on creatine excretion, and as no figures for urinary nitrogen are included comparison with our results is difficult.

EXPERIMENT II.

Subject 2. Male, 13 Years, Weight 29 Kilos.

Date	24 hour volume.	Creatinine.	Creatine.	Total nitrogen.	Sodium bicarbonate taken.
1918	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*
June 9–10	1,400	0.84	0.21	10.64	0
" 10–11	1,560	0.74	0.07	6.70	0
" 11–12	2,000	0.94	0.16	7.50	10
" 12–13	1,640	0.80	0.12	6.80	10
" 13–14	1,860	0.76	0.15	5.95	10
" 14–15	1,460	0.71	0.20	5.50	10
" 15–16	1,280	0.70	0.16	6.08	0
" 16–17	940	0.71	0.12	5.82	0
" 17–18	890	0.79	0.19	6.49	0

EXPERIMENT III.

Subject 3. Female, Hyperthyroidism, 32 Years, Weight 45 Kilos.

Date.	24 hour volume.	Creatinine.	Creatine.	Total nitrogen.	Sodium bicarbonate taken.
1918	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*
Feb. 2– 3	1,020	0.72	0.35	11.32	0
" 3– 4	1,400	0.70	0.56	15.89	0
" 4– 5	2,260	0.72	0.50	18.64	15
" 5– 6	1,780	0.72	0.39	14.70	15
" 6– 7	1,510	0.75	0.21	13.21	15
" 7– 8	1,400	0.75	0.65	13.89	0
" 13–14	1,220	0.76	0.25	11.20	0
" 14–15	1,020	0.76	0.24	11.30	0
" 15–16	1,800	0.72	0.48	14.40	15
" 16–17	1,480	0.69	0.23	8.65	15
" 17–18	1,900	0.70	0.24	10.54	15

The great variations in the daily figures for preformed creatinine contained in this work, variations in some cases amounting to 400 per cent, and the extremely low figures for preformed creatinine given for adult women, make it seem possible that some

EXPERIMENT IV.

Subject 4. Female, Hyperthyroidism, 22 Years, Weight 41 Kilos.

Date.	24 hour volume.	Creatinine.	Creatine.	Total nitrogen.	Sodium bicarbonate taken.
1918	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*
Feb. 27–28	900	0.60	0.45	11.78	0
" 28–Mar. 1	780	0.58	0.42	11.46	0
Mar. 1–2	—	—	—	—	0
" 2–3	840	0.58	0.31	11.59	15
" 3–4	1,080	0.58	0.18	9.72	15
" 4–5	1,320	0.66	0.22	10.95	15
" 5–6	1,380	0.65	0.18	11.51	15
" 6–7	1,340	0.60	0.17	9.64	0
" 7–8	1,180	0.72	0.18	10.62	0

EXPERIMENT V.

Subject 5. Female, Hyperthyroidism, 28 Years, Weight 51 Kilos.

Date.	24 hour volume.	Creatinine.	Creatine.	Total nitrogen.	pH	Sodium bicarbonate taken.
1918	*cc.*	*gm.*	*gm.*	*gm.*		*gm.*
Apr. 27–28	1,160	0.50	0.43	11.60	5.1	0
" 28–29	1,140	0.50	0.44	11.70	5.3	0
" 29–30	1,160	0.46	0.43	12.08	5.3	10
" 30–May 1	1,250	0.62	0.65	15.8	7.0	15
May 1–2	1,240	0.49	0.40	10.29	7.0	15
" 2–3	1,600	0.56	0.44	12.64	7.0	15
" 3–4	1,150	0.61	0.38	10.69	7.0	0

EXPERIMENT VI.

Subject 6. Female, Hyperthyroidism, 27 Years, Weight 63.3 Kilos.

Date.	24 hour volume.	Creatinine.	Creatine.	Total nitrogen.	pH	Sodium bicarbonate taken.
1918	*cc.*	*gm.*	*gm.*	*gm.*		*gm.*
Apr. 28–29	1,140	0.70	0.88	19.72	5.1	0
" 29–30	1,040	0.73	0.94	20.59	5.7	10
" 30–May 1	1,380	0.60	0.55	13.66	6.3	15
May 1–2	2,140	0.81	0.49	19.68	7.0	15
" 2–3	1,720	0.67	0.55	17.71	7.0	15
" 3–4	1,600	0.81	0.32	15.20	6.2	0
" 4–5	1,360	0.68	0.65	15.09	5.0	0
" 5–6	1,440	0.80	0.88	18.14	5.0	0
" 6–7	1,280	0.66	0.66	16.00	5.3	0

of the experimental subjects could scarcely be classed as normal. For instance in Experiment I a figure of 85 mg. of preformed creatinine is given as the amount of this substance excreted on February 23, 1915, by a woman weighing 60 kilos. 6 days later, March 1, the same subject excreted 162 mg. of preformed creatine, and on March 4, 356 mg. As a common figure for preformed creatinine excretion in 24 hours for infants of 1 year weighing about 10 kilos is in the neighborhood of 100 mg., it is difficult to connect some of the above figures with normal adult women.

EXPERIMENT VII.

Date.	24 hour volume.	Creatinine.	Creatine.	Total nitrogen.	Sodium bicarbonate taken.
1918	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*
Jan. 21–22	1,220	1.14	0	6.0	0
" 22–23	1,060	1.16	0.12	—	0
" 23–24	1,160	1.15	0.07	—	0
" 24–25	1,180	1.21	0.18	—	0
" 25–26	960	1.03	0.32	13.49	0
" 26–27	1,280	1.23	0.59	21.63	0
" 27–28	1,060	1.26	0.38	17.28	10
" 28–29	960	1.07	0.27	17.37	15
" 29–30	1,080	1.18	0.41	19.98	15
" 30–31	1,500	1.57	0.30	21.75	15

Subject 7.—Normal female, 39 years, weight 90 kilos. Jan. 21 to 22, a low protein diet free from meat was taken. On Jan. 22, a high protein diet consisting of the following foods was started and continued to the end of the experiment: bread 200 gm., butter 50 gm., twelve eggs, gelatin 50 gm., cheese 200 gm., two oranges, two lemons. This diet contains approximately 29 gm. of nitrogen and 2,200 calories.

Rose, Dimmitt, and Bartlett[5] have also published the results of experiments on the subject of high protein feeding which have caused them to come to the conclusion that "the ingestion of diets excessively high in protein fails to induce the excretion of creatine in normal women and men." In connection with this conclusion we would call attention to the fact that the two experiments on women made by these investigators are not in any way a repetition of our experiments on this subject. Thus in their

[5] Rose, W. C., Dimmitt, J. S., and Bartlett, H. L., *J. Biol. Chem.*, 1918, xxxiv, 601.

experiments the highest diet taken by the female subject A.B. contained 21.76 gm. of nitrogen and 2,243 calories per day, while the female subject E. B. took only 16.16 gm. of nitrogen and 1,759 calories; furthermore, these diets were taken for a period of 5 days only and followed a period of in one case 6 and the other 5 days during which a low protein diet had been consumed.

EXPERIMENT VIII.

Date.	24 hour volume.	Creatinine.	Creatine.	Total nitrogen.	Sodium bicarbonate taken.
1918	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*
Jan. 17–18	1,020	1.19	0	5.2	0
" 18–19	1,220	1.13	0	—	0
" 19–20	1,060	1.16	0.08	—	0
" 20–21	980	0.99	0.06	—	0
" 21–22	1,020	1.10	0	—	0
" 22–23	1,200	1.19	0.17	—	0
" 23–24					
" 24–25	1,080	1.08	0.20	16.60	0
" 25–26	1,030	1.00	0.28	17.32	0
" 26–27	1,110	1.22	0.38	21.78	0
" 27–28	1,300	1.01	0.30	23.63	0
" 28–29	1,320	1.13	0.11	23.49	10
" 29–30	1,120	1.11	0.14	20.49	15
" 30–31	1,160	1.16	0.11	19.25	15
" 31–Feb. 1	1,170	0.99	0.12	18.25	15
Feb. 1–2	1,120	1.12	0.18	20.16	0
" 2–3	1,540	1.05	0.15	21.16	0

Subject 8.—Normal female, 23 years, weight 53 kilos. Jan. 17 to 18 was the last day of an 8 day period of low protein feeding. On Jan. 18, a period of high protein feeding was started and continued to the end of the experiment. The daily ration consisted of the following: bread 150 gm., butter 25 gm., milk 500 cc., twelve eggs, gelatin 55 gm., cheese 200 gm., string beans (canned) 100 gm. This diet contains approximately 31 gm. of nitrogen and 2,200 calories.

In the experiments which justified our statement that creatinuria could be produced in women by high protein feeding, the daily ration contained approximately 33 gm. of nitrogen, an amount a little more than twice that contained in the diet of the second female subject of Rose, Dimmitt, and Bartlett. Furthermore, we have found by experience, that in the production of

creatinuria by forced protein feeding, the desired result is obtained more slowly if the preceding diet has been of a low protein nature.

The two normal women who were used in our experiments of last year were again used in Experiments VII and VIII of this paper, and as will be seen it took several days of high protein feeding before creatinuria was induced. It is to be admitted that more experimental work on a larger number of women is necessary before a decision can be reached in this matter. It is, however, extremely difficult to obtain female subjects possessing sufficient physical courage and scientific enthusiasm to carry them through such a disagreeable dietary experiment, and, as after a considerable number of trials, we have been unable to obtain further experimental material our first paper was published in the hope that our experiments might stimulate sufficient interest to cause them to be repeated in other laboratories.

THE ANTISCORBUTIC PROPERTY OF VEGETABLES.

I. AN EXPERIMENTAL STUDY OF RAW AND DRIED TOMATOES.*

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(Received for publication, December 18, 1918.)

The following experiments are a part of a large series planned to determine the antiscorbutic potency of different foods, the effect of preparation and preservation upon these foods, and other similar questions. Desiccation of certain foods, particularly vegetables, as a means of preservation, has been advocated widely because of war conditions. There can be no question but that such a procedure is justified from an economic standpoint, but concerning the physiological value of desiccated foods we need enlightenment. Givens and Cohen[1] have reported an experimental study of dried cabbage. This work on dried vegetables we have continued at the request of the Division of Food and Nutrition, Medical Department, U. S. Army. It is of great importance to have all possible information concerning foods accessible for incorporation into the dietary of our soldiers and civilians.

There has been a difference of opinion as to the existence of a third type of vitamine, one protecting against scurvy. From the work of Holst and Frölich,[2] Chick and Hume,[3] Hess and Unger,[4] Harden and Zilva,[5] Cohen and Mendel,[6] and Givens and

* A preliminary report of this work was given at the meeting of the Society for Experimental Biology and Medicine, Oct. 16, 1918.

[1] Givens, M. H., and Cohen, B., *J. Biol. Chem.*, 1918, xxxvi, 127.

[2] Holst, A., and Frölich, T., *Z. Hyg.*, 1912, lxxii, 1; 1913, lxxv, 334.

[3] Chick, H., and Hume, M., *Tr. Soc. Trop. Med. and Hyg.*, 1916–17, x, 141.

[4] Hess, A. F., and Unger, L. J., *J. Biol. Chem.*, 1918, xxxv, 479, 487.

[5] Harden, A., and Zilva, S. S., *J. Inst. Brewing*, 1918, xxiv, 197; *Biochem. J.*, 1918, xii, 93.

[6] Cohen, B., and Mendel, L. B., *J. Biol. Chem.*, 1918, xxxv, 425.

Cohen,[1] there is evidence of the presence in certain foods of some substance which behaves like a vitamine by affording protection against experimental scurvy in the guinea pig.

It is widely recognized that scurvy can be prevented or cured by the consumption of fresh vegetables. To locate citations directing the use of specific vegetables and experimental evidence for that use is another question. We are able to find only one direct statement bearing on the use of raw tomatoes as antiscorbutic material. Munson[7] states that "the raw potato, onion, and tomato are especially valuable" in the prophylaxis of scurvy. The tomato has long enjoyed the status of an unessential accessory article of diet, its value being attributed to its flavor. That it can play the rôle of an essential accessory in preventing the development of scurvy, we believe the following experiments will show.

There has not been an extensive use of dried vegetables as antiscorbutic agents. Presumably the reasons for this are twofold. In the first place, until lately only a few dehydrated foods for human consumption have been on the market. Secondly, notwithstanding propaganda to stimulate the use of desiccated foods, particularly green stuffs, there has been offered very little scientific evidence guaranteeing the presence in the dried materials of the original properties of the raw. In fact, the majority of such evidence which has been offered regarding the antiscorbutic potency of dried foods has been of a negative character.[2, 3] If it can be physiologically demonstrated that desiccated foods retain all their original properties, then the case of preservation by dehydration is complete and justified.

Holst and Frölich,[2] and Chick and Hume[3] found dried vegetables more or less deficient in the antiscorbutic property. Recently Chick, Hume, and Skelton[8] have reported, with other interesting data, that the feeble antiscorbutic property of fresh milk is destroyed either by drying or the long keeping after drying. Hess and Unger[4] have stated that "dehydrated vegetables were found to contain little or no antiscorbutic virtue."

[7] Munson, E. L., The theory and practice of military hygiene, New York, 1901, 762.

[8] Chick, H., Hume, E. M., and Skelton, R. F., *Biochem. J.*, 1918, xii, 131.

They draw this conclusion from the fact that in certain experiments guinea pigs were not protected from scurvy by eating dried carrots. Stefánsson[9] alleges that "the strongest antiscorbutic qualities reside in certain fresh foods and diminish or disappear with storage by any of the common methods of preservation—canning, pickling, drying, etc." His statement is based upon his experience with such products used as rations for men on polar expeditions.

Givens and Cohen[1] have reported experiments dealing with the influence of temperature on the antiscorbutic vitamine in the drying of cabbage. It is their opinion, as regards this vegetable, that the degree of temperature in drying is the factor which determines the absence or presence of the accessory scurvy-protecting substance. Most of their dried material was used within a month after its preparation, consequently no evidence was offered as to the effect of aging. This point will be reported upon in the future. In the following experiments the influence of temperature on the antiscorbutic property of the tomato is shown. The selected temperatures at which the drying was carried out also permit certain conclusions regarding the effect of length of time of drying. The problem of the potato is more complicated than is indicated by the report of Givens and Cohen,[1] consequently it will be dealt with in detail in a future communication.

Drier and Drying of Tomatoes.

Because of the lack of available, accurate information on the best mode and temperature for drying foods, we constructed a special apparatus.[10] Accordingly a drier has been manufactured which permits us to control the temperature within 5° or less, at all times. The volume of air is always the same. The humidity of the entering air depends on atmospheric conditions. However, the ingoing air can be dried if such a procedure appears necessary.

The apparatus is made of galvanized metal and is covered with asbestos. In the sides are drawers, having framework only for

[9] Stefánsson, V., *J. Am. Med. Assn.*, 1918, lxxi, 1715.

[10] Our thanks are due Mr. J. F. Barker, President of Mechanics Institute, Rochester, for designing and directing the construction of the drier.

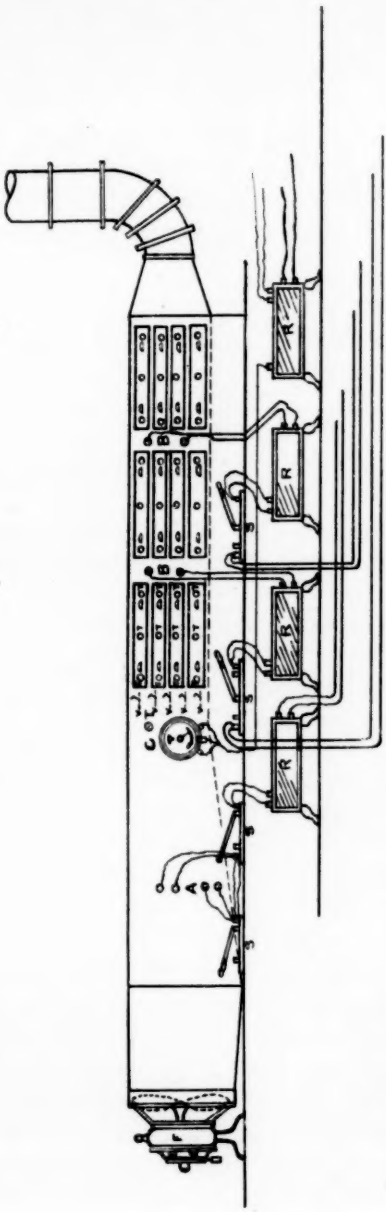
their sides and with the bottoms made of wire netting, ¼ inch mesh. Drawers so constructed permit a free circulation of air through all parts. In the front end of the machine is located a 12 inch fan. Midway between the fan and the first section of drawers a number of electric heating coils are placed. The entering air is thus heated before it strikes the first section of drawers. After each section of drawers there are supplementary electric coils. These coils bring the moving air, which has been slightly cooled as it passed over the first section, up to its former temperature. A thermostat controls the heating current. The temperature of each drawer can be determined at any time by the two thermometers inserted. The details of the drier are shown in Fig. 1.

Tomatoes dried at two ranges of temperature have been used; "low-dried," 35–40°C., and "high-dried," 55–60°C. The maximum and minimum times of drying for the "low-dried" tomatoes were 52 and 35 hours respectively; for the "high-dried," 26 and 13 hours respectively. The variations in the time of drying are caused partly by the difference in water content of various samples of tomato and partly by the variable moisture content of the air. None of the tomatoes were dried without interruption. The process had to be stopped at the end of each day and was taken up on the next.[11]

The tomatoes were wiped clean with a cloth, stems removed, and bad spots, if any, excised. They were then cut into pieces shaped like a pyramid, the skin forming the base, and placed so on the mesh bottom drawers of the drier. A slight amount of juice was lost in the cutting, but since the operation was done in pans, the juice was weighed, and corresponding corrections made for the percentage of the dried product in terms of the raw. The tomatoes were kept in the drier until there was no further loss in weight in the course of an hour's drying. The dried products were kept in ordinary glass-capped, rubber-ringed preserving jars. Details concerning the dehydrated products, with the time elapsing between drying and using, are given in Table I.

The high-dried product differed from the low-dried in that it was subjected to 20°C. higher temperature and the time of drying

[11] We are indebted to Misses Van Horne and Venor, of Mechanics Institute, for drying under our direction the materials used in these experiments.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reduced. Also it contained slightly less water. All samples of dried tomatoes were fed as such, the daily allowance of this supplement being 1 gm. per animal. The animals ate greedily this allotment.

Raw tomatoes were always given in the amount of 10 gm. per animal per day. They were wiped off with a damp cloth and sliced so that each animal received approximately the same proportion of skin, pulp, and seed. Guinea pigs relish raw tomatoes.

TABLE I.

Lot No.	Sample No.	Date dried.	Date started using.	Date finished using.	Duration of drying.	Per cent of original weight.
Low-dried tomatoes, 35–40°C.						
		1918	*1918*	*1918*	*hrs.*	
XXIII	1, 2	Aug. 15	Aug. 21	Sept. 13	$36\frac{1}{2}$	6–7
31	1	" 23	Sept. 13	Oct. 3	$35\frac{3}{4}$	6.5
34	2	" 29	Oct. 4	" 16	$38\frac{3}{4}$	7.3
31	2	" 23	" 17	" 29	$35\frac{3}{4}$	6.7
37	1	" 30	" 29	Nov. 7	$42\frac{1}{2}$	7.5
34	3	" 29	Nov. 8	" 18	$38\frac{3}{4}$	7.3
High-dried tomatoes, 55–60°C.						
XVIII	2	July 31	Aug. 1	Aug. 24	23	6.5
	1	" 31	" 25	Sept. 25	23	6.5
XX	1	Aug. 3	Sept. 26	Oct. 12	14	6.5
	2	" 3	Oct. 13	" 26	14	6.5
XI	1	" 7	" 27	Nov. 1	$14\frac{1}{2}$	7.1
XXII	—	" 8	Nov. 2	" 5	14	7.2
38	3	Sept. 12	" 6	" 16	$24\frac{3}{4}$	6.8
39	1	" 13	" 17	" 18	$25\frac{3}{4}$	7.3

Diet.

The basal diet was a dried product containing soy bean flour, milk, yeast, paper pulp, sodium chloride, and calcium lactate. It was made according to the directions given by Givens and Cohen.[1] In certain cases more of the heated soy flour has been added to the diet; the specific instances are noted in Table II.

In the light of our present knowledge of the so called biological properties of foods, based on experiments conducted on the rat, the diet of oats alone used to produce scurvy in guinea pigs can

be criticised. McCollum and Pitz[12] allege that oats are deficient in adequate proteins, inorganic salts, and their fat-soluble A. If we accept their standard for a complete diet, then the experiments of Cohen and Mendel,[6] of Givens and Cohen,[1] and the present work, on scurvy produced experimentally in the guinea pig on a soy bean mixture, are the only ones in which the diet was complete. This diet contains adequate protein, sufficient fat, carbohydrates, and inorganic salts, and a liberal supply of fat- and water-soluble vitamines. This statement is based on the fact that Givens and Cohen were able to induce normal growth in rats supplied this diet *ad libitum*. However, these experiments are open to the criticism that in supplying the diet *ad libitum* to the rats, they obtained more of the vitamines than would have been the case if the food intake had been limited as it was with the guinea pigs. That such a criticism is not valid we believe from the following experiments conducted on pigeons.

Two pigeons have been fed on the same basal soy cake mixture which produces scurvy in the guinea pig. Their daily supply of this food has been limited to the same weighed amount received daily by the guinea pigs. The actual daily intake, unless otherwise noted, of the guinea pigs and the birds was approximately 11.1 gm. of soy flour, 14.0 cc. of whole milk, 0.44 gm. of dried yeast, 1.1 gm. of filter paper, 0.44 gm. of calcium lactate, and 0.44 gm. of sodium chloride. The birds ate very little of the food mixture at the beginning of the experiment. Beginning with the 16th day and continuously since, they have consumed all the food allotted per day. These birds are now perfectly healthy after receiving the soy cake mixture for 105 days (Fig. 2). They have never shown any signs of polyneuritis. That they should drop in weight at first and not regain at once their initial weight was to be expected when they did not eat, and when the nutrients fed were limited and no doubt insufficient in amount to meet their metabolic requirements. However, the experiments confirm the results obtained on feeding the soy cake mixture to rats, in that they demonstrate conclusively that the soy cake mixture contains a sufficient amount of the water-soluble vitamine to

[12] McCollum, E. V., and Pitz, W., *J. Biol. Chem.*, 1917, xxxi, 229.

prevent polyneuritis in the pigeon—a species particularly susceptible to this disease. That the diet contains enough of the fat-soluble vitamine also we believe has been demonstrated by the work of Cohen and Mendel and of Givens and Cohen. This assumption is further supported by the results reported by Osborne and Mendel[13] and by Daniels and Nichols.[14] In the soy cake mixture used in these experiments, the amount of fat-soluble vitamine in the soy flour has been augmented by the addition of whole milk. Bulk in the diet has been provided in the form of paper pulp. The inorganic constituents have been supplemented by the addition of sodium chloride and calcium lactate. In the light of the present available evidence, we feel justified in concluding that the diet used to produce scurvy in guinea pigs in these experiments is adequate in every respect, with the one exception of being deficient in a vitamine protecting against scurvy. Such a diet removes the possibility of the influence of any deficiencies other than the antiscurvy vitamine.

General Procedure and Methods.

All the guinea pigs used were young ones. In general we tried to obtain them at about 8 weeks of age. They were the offspring of strong, healthy stock. Reliable parties conducted the breeding and rearing of the animals and kept us informed regarding the manner in which they were handled. The food consumed by the guinea pigs prior to starting the experimental diet was varied, and contained plenty of green foods. We mention these facts because a properly reared animal is much stronger and more healthy than one treated in a haphazard way.

When an experiment was started, each animal was placed in a separate metal cage and kept there alone at all times. Once daily, weighed amounts of food were supplied in individual cups. Tap water was give *ad libitum*. The animals were examined every day and weighed every other day. In this way the first appearance of the specific symptoms of scurvy were noted.

Autopsies were performed as early as possible after death. Animals in a moribund condition were usually anesthetized and

[13] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1917, xxxii, 369.
[14] Daniels, A. L., and Nichols, N. B., *J. Biol. Chem.*, 1917, xxxii, 91.

examined at once. A decision of scurvy was based upon clinical manifestations and the usual autopsy findings (Givens and Cohen,[1] Chick, Hume, and Skelton[8]). Of course, when curative measures were instituted and the animal saved, scurvy was diagnosed from the usual external symptoms and that diagnosis confirmed by autopsies on control animals. The results are shown in Table II and Figs. 3 to 6.

Results.

For control experiments on the soy cake mixture and this mixture plus a small supplement of green food, as cabbage, reference is made to the charts of Givens and Cohen. These workers found that guinea pigs on the soy cake mixture *alone* regularly developed scurvy and succumbed in 16 to 26 days unless curative measures were instituted.

Experiments with Raw and Dried Tomatoes.

In the present experiments raw tomatoes have been used both as a preventive and curative agent in experimental scurvy of the guinea pig. Group 1 (Table II and Fig. 3) demonstrates conclusively that a daily supplement of 10 gm. of raw tomatoes will afford a guinea pig protection against scurvy when that animal is on a diet known to produce the disease in this species. These animals have grown and been maintained in apparently perfect health for a period of 110 days. If the supplement of raw tomatoes had not been given, these animals, as has been proved, would have died of scurvy in 16 to 26 days. That the tomato will serve equally well as a curative is shown by Group 2 (Table II and Fig. 4). These animals developed signs of scurvy in 18 to 19 days. After a few days, each guinea pig was fed daily a supplement of 10 gm. of raw tomatoes. They responded to the treatment in a few days, became lively, their symptoms of scurvy gradually disappeared, and they began to increase in weight. They are now, at 100 days, large, healthy animals. In Guinea Pig 227 (Fig. 3), which died on the 31st day of the experiment, no macroscopic signs of scurvy were present.

Group 3 (Table II and Fig. 5) represents a series of experiments which demonstrate that tomatoes dried at a low temperature

TABLE II.

Group and diet.	No.	Weight. Initial.	Weight. Maximum	Weight. Final.	Duration of experiment.	Remarks.
		gm.	*gm.*	*gm.*	*days*	
1						
Soy diet + 10 gm. raw tomatoes daily.	227	157	225	192*	30	Cause of death unknown. No signs of scurvy.
	228	150	374	374	110	Never any signs of scurvy.
	229	199	410	410	110	" " " " "
	239	160	390	390	90	" " " " "
2						
Soy diet alone until scorbutic; then daily supplement of 10 gm. raw tomatoes.	237	142	364	364	100	Symptoms appeared on 19th day. Supplement of 10 gm. raw tomatoes daily beginning on 20th day. Cured of scurvy.
	238	148	322	322	100	Symptoms appeared on 15th day. Supplement of 10 gm. raw tomatoes daily beginning on 18th day. Cured of scurvy.
3						
Soy diet + 1 gm. of low-dried (35–40°) tomatoes raw.	240	106	280	270*	74	Cause of death anatomical anomaly. No signs of scurvy.
	241	167	300	290	92	Never any signs of scurvy.
	242	137	230	198*	72	Cause of death unknown. No signs of scurvy.
	243	136	308	296	92	Never any signs of scurvy.
	244	172	320	320	92	" " " " "
4						
Soy diet + 1 gm. high-dried (55–60°) tomatoes raw.	230	164	374	374	110	Never any signs of scurvy.
	231	176	388	388	110	" " " " "
	232	172	436	436	110	" " " " "

* Weight at death.

(35–40°C.) retain some of the original antiscorbutic potency of the raw tomato. A supplement of 1 gm. daily of this dried material has been effective in protecting these animals for 92 days.

Group 4 (Table II and Fig. 6) shows that tomatoes dried at a temperature of 55–60°C. also retain some of the antiscorbutic vitamine. A daily supplement of 1 gm. of this material has afforded these animals protection against scurvy for 110 days.

DISCUSSION.

It has been established that a diet apparently adequate for the rat and the pigeon will produce scurvy in the guinea pig. If this diet is supplemented with a daily allowance of 10 gm. of raw tomatoes, no signs of scurvy will develop in the latter species. This property of raw tomato is also effective as a curative agent, yielding results similar to those obtained with raw orange juice. Therefore it seems demonstrated that the tomato may be considered to contain some of the essential dietary accessory substance.

Any discussion of the results obtained with dried tomatoes must be governed by the experimental procedures employed. Only one kind of drying has been employed—hot air. The temperatures at which the products were dried differed by a range of 20°C., consequently there is a difference in the duration of drying. It might be conceived that a longer duration of drying at a low temperature was more destructive than a short duration at a high temperature. No light is thrown upon this point in the present experiments because it has not been established what minimum amount is necessary of either the low- or high-dried materials barely to protect a guinea pig against scurvy.

The dried products used here permit of some consideration of the possible influence of aging. The low-dried tomatoes were still effective as antiscorbutic agents 80 days after drying; the high-dried, 90 days. This fact, however, does not preclude the possibility of some destruction or spontaneous disintegration of the antiscorbutic vitamine. Until there has been worked out a method for determining quantitatively the amount of this vitamine in a food, little that is definite can be said regarding the effect of aging in a dried product which retains some of its antiscurvy vitamine. It might be assumed that there was contained in 1

gm. of the dried tomatoes an excess of the accessory above the amount necessary to protect the given body weight of animals used. Then this excess could have been destroyed in the aging and still we should have encountered no signs of scurvy. At any rate it is logical to conclude that tomatoes dried in the manner here described retain some of their antiscorbutic potency after storage of 3 months.

Pitz[15] has offered experiments to show that sodium chloride and calcium lactate afforded guinea pigs some protection against scurvy, and therefore delayed the appearance of scorbutic signs. Our basal diet contained more of both of these salts than the one he used. We obtained scurvy in our animals in a very short time, indicating no favorable influence of the salts when present in the soy flour-milk-yeast-paper mixture. However, we concur in the opinion expressed by Hess and Unger that it is impossible to interpret contradictory results when dissimilar basal dietaries are used. The results with low-dried tomatoes confirm those obtained by Givens and Cohen with cabbage dried at a low temperature. No comparison can be made between the findings with high-dried cabbage and high-dried tomatoes. The former were dehydrated at a higher temperature and were subjected to a different kind of heat; *viz.*, moist heat. Holst and Frölich report that the antiscorbutic substance in food is very easily destroyed by high temperatures with moisture present.

SUMMARY.

Experiments have been carried out which show that the raw fresh tomato is a very efficient antiscorbutic agent. A small daily addition of this substance to a diet known to produce scurvy in guinea pigs maintained these animals in apparently perfect health. It is here demonstrated that raw fresh tomatoes dried in a blast of air at either a low temperature (35–40°C.) or a high temperature (55–60°C.) retain *a significant amount* of their antiscorbutic potency. It is possible that the drying at either temperature may destroy some of the antiscorbutic vitamine but to what degree cannot be stated yet. A daily supplement of 1 gm. of raw dried tomatoes protected guinea pigs from experimental scurvy.

[15] Pitz, W., *J. Biol. Chem.*, 1918, xxxvi, 439.

These experiments lend confirmation to the results of Chick, Hume, and Skelton who believe "that guinea-pig scurvy is due to the deficiency in the diet of a specific accessory substance." They support the contention of Cohen and Mendel that roughage, as it affects the texture of the diet, is not the determining factor.

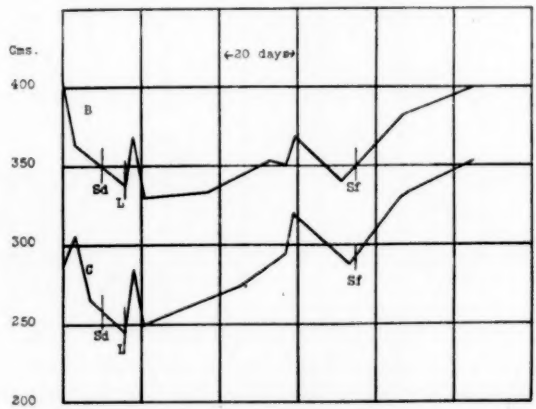

FIG. 2. Curves of body weights of two pigeons fed on the basal scurvy-producing diet. The curve of Pigeon B is moved 50 gm. above actual weights to keep it from cutting the other curve. From the 10th day on the birds were supplied with sand and gravel (Sd). At first the pigeons did not eat all of the soy cake mixture. By the 16th day they had acquired a taste for it. From this time until the 75th day they received the same limited amount of it (L) as fed to the guinea pigs. From the 75th day on the daily intake was supplemented (Sf) with 5 gm. of heated soy bean flour. Their response to the increased food was immediate. These curves show that the diet which produces scurvy in the guinea pig is adequate for the pigeon and therefore contains enough of the water-soluble vitamine to prevent polyneuritis.

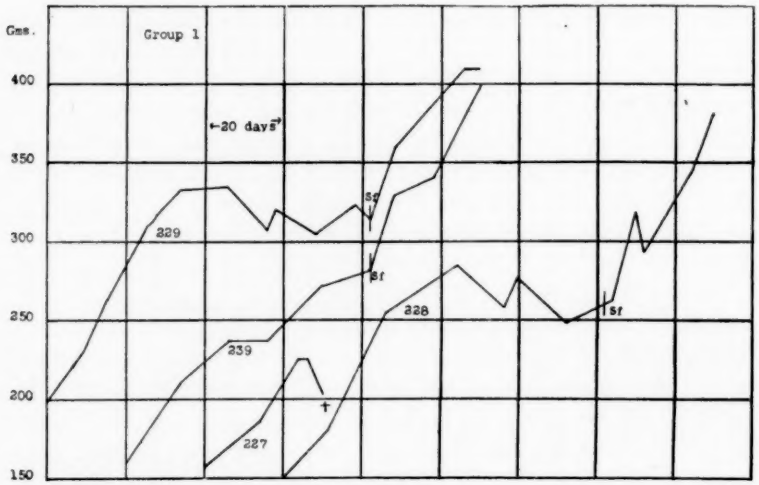

FIG. 3. Group 1. The guinea pigs in this group received daily a limited amount of the soy cake mixture and 10 gm. of raw fresh tomatoes. Animal 227 died after 30 days, showing at autopsy no macroscopic signs of scurvy. At the end of 50 days Animals 228 and 229 had apparently reached a body weight which could only be maintained and not increased by the limited food intake. To be sure that it was not the appearance of scurvy, no alterations in the diet were made until the 82nd day. At this time an addition of 5 gm. of soy bean flour (Sf) heated at 20 pounds pressure for 30 minutes was made to the diet. The response to this increased food intake was immediate. In the case of Animal 239 the heated soy flour (Sf) was added on the 62nd day and there was likewise a prompt growth response. These experiments show that the raw fresh tomato is a strong antiscorbutic agent.

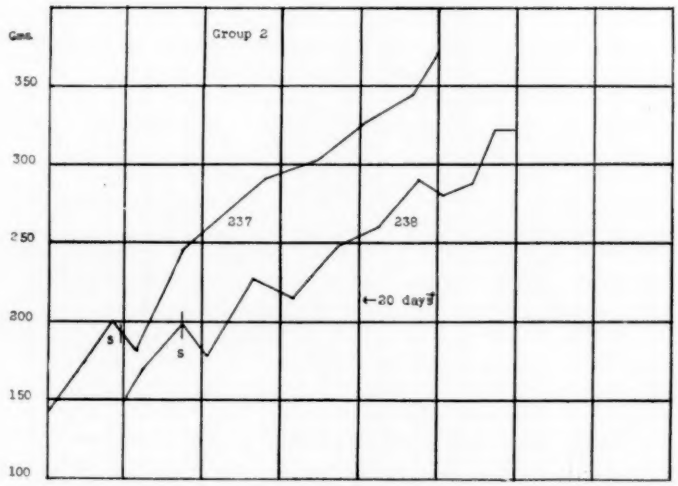

FIG. 4. Group 2. These experiments demonstrate that the raw tomato is equally effective as a curative or a preventive agent. Animal 237 on the soy cake mixture alone showed definite signs of scurvy on the 19th day. Beginning with the 20th day it received a daily supplement of 10 gm. of raw tomatoes. Animal 238 on the basal scurvy-producing diet developed positive symptoms of scurvy on the 15th day. From the 20th day on it received a daily addition of 10 gm. of raw tomatoes. Both guinea pigs responded promptly to the dietary change, becoming lively, eating better, and increasing in weight. The appearance of the first signs of scurvy, swollen and tender joints, is noted in the chart at S.

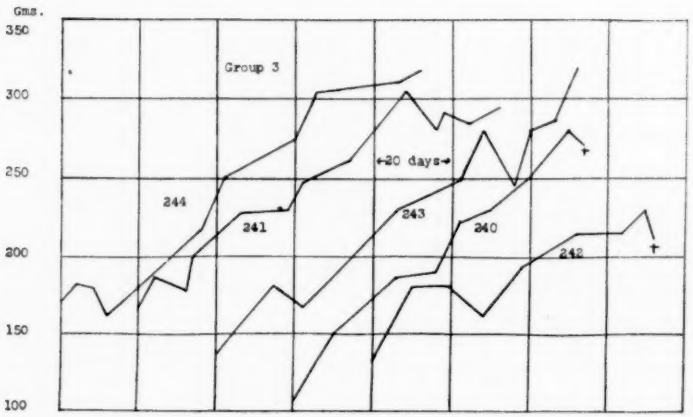

FIG. 5. Group 3. This group shows the antiscorbutic value of tomatoes dried at a low temperature (35–40°C.). A daily supplement of 1 gm. of this dried raw material uncooked has protected three of the guinea pigs during 94 days. That the limited food intake is not sufficient for their present body weight is indicated by the way two of them are just maintaining their weight. Animal 240 died on the 74th day as a result of an anatomical anomaly. It showed at autopsy no macroscopic signs of scurvy. Animal 242 died on the 72nd day from an unknown cause, showing no indication of scurvy.

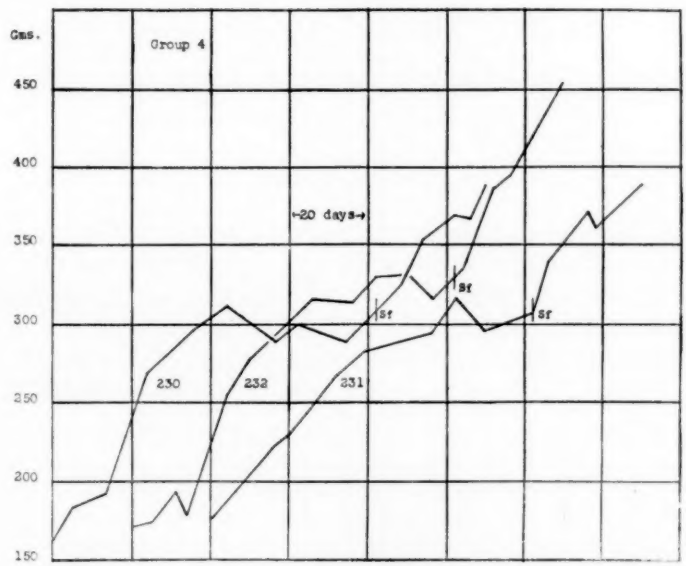

FIG. 6. Group 4. This series demonstrates that raw tomatoes dried at a comparatively high temperature still retain a significant amount of the antiscorbutic substance. A daily supplement of 1 gm. of high-dried (55–60°C.) tomatoes has protected these guinea pigs against scurvy for 110 days,—a period four to seven times as long as that in which scurvy would have developed in the absence of the supplement. It became evident after 50 days that the animals were not receiving enough food to meet their requirements. On the 82nd day 5 gm. of heated soy flour (Sf) were added daily to the food intake. The immediate response is shown by gain in weight.

THE PHYSICOCHEMICAL STATE OF THE PROTEINS IN COW'S MILK.*

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INTRODUCTION.

Both qualitative and quantitative investigations regarding the proteins of cow's milk based on what may be called a mechanical separation of the several constituents date back a number of years. With the exception of Hoppe-Seyler,[1] who used an animal membrane, all the workers in this field have made use of porous clay in some form.

Herman Helmholz had his pupil Zahn[2] construct a filter out of a porous porcelain cylinder, and this form of apparatus was also used by Kehrer.[3] Hermann[4] modified Zahn's method and used powdered porous plate to adsorb the casein. Tiemann,[5] however, several years later made extensive use of a method devised by Lehmann,[6] in which the milk was placed upon a slightly concave, porous plate, whose surface had been polished with agate. After standing for 1½ to 2 hours, the unadsorbed part, consisting of casein and fat, was scraped off and weighed. Tiemann applied this method especially to the study of the proteins of colostrum milk. Camerer and Söldner,[7] however, have criticized Tiemann's method of analysis on the ground of the probable lack of uniformity of clay filters and also because they do not regard the casein to be completely retained on the porous plate.

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* Published by permission of the Director of the Missouri Agricultural Experiment Station.

[1] Hoppe-Seyler, F., *Virchows Arch. path. Anat.*, 1859, xvii, 421–423.

[2] Zahn, F. W., *Arch. ges. Physiol.*, 1869, ii, 598.

[3] Kehrer, F. A., *Arch. Gynäk.*, 1871, ii, 5 (cited by Schlossmann[17]).

[4] Hermann, L., *Arch. ges. Physiol.*, 1881, xxvi, 442.

[5] Tiemann, H., *Z. physiol. Chem.*, 1898, xxv, 363.

[6] Hempel, W., *Arch. ges. Physiol.*, 1894, lvi, 558.

[7] Camerer and Söldner, *Z. Biol.*, 1898, xxxvi, 277.

Van Slyke and Bosworth[8] have recently made use of what they regard as a much superior form of apparatus for mechanically separating the constituents of milk, by means of which they hoped to ascertain the true state of the proteins and salts in milk. For this purpose they used a special form of Briggs'[9] filtering apparatus, designed to obtain water extracts from soils. Van Slyke and Bosworth describe the procedure as follows:

"The process consists in putting the milk to be examined into a tubular chamber surrounding a Pasteur-Chamberland filtering tube; pressure, amounting to 40 to 45 pounds per square inch, is applied by means of a pump which forces air into the chamber containing the milk and causes the soluble portion of the milk to pass through the walls of the filter from the outside to the inside of the filtering tube, from which it runs out and is caught in a flask standing underneath."

The portion of Van Slyke and Bosworth's study of cow's milk which particularly attracted our attention and caused us to carry out the experiments presented in this paper was the conclusion that the albumin of cow's milk is partly in true solution and partly in suspension, the suspended portion being adsorbed by the casein when the milk is fresh but passing over into true solution when the milk sours or when formaldehyde is added to prevent bacterial development. These statements have been accepted as facts by certain authors.[10]

Our study of this question, both from a theoretical and from an experimental point of view has failed to lead to a confirmation of Van Slyke and Bosworth's conclusions regarding the condition of albumin in cow's milk. Our work did not have to do with the state of casein in milk, as the evidence which has already accumulated warrants the conclusion that it is present in fresh milk wholly in a colloidal state. Its degree of dispersion is, moreover, relatively coarse, inasmuch as cow's milk owes its familiar "milky white" appearance almost wholly to casein.

On the other hand, we feel justified in asserting that the filtration of lactalbumin through a particular Pasteur-Chamberland filtering tube, or its failure to do so is not the proper criterion by which to judge whether it is in a colloidal or true state of

[8] Van Slyke, L. L., and Bosworth, A. W., *N. Y. Agric. Exp. Sta., Technical Bull. 39*, 1914; *J. Biol. Chem.*, 1915, xx, 136.

[9] Briggs, L. J., *U. S. Dept. Agric., Bureau of Soils, Bull. 19*, 1902, 31.

[10] Hawk, P. B., Practical physiological chemistry, Philadelphia, 5th edition, 1916, 313.

solution in milk. Not only does the size of the pores of the Pasteur-Chamberland tube vary,[11] but the size of the lactalbumin particles may be influenced greatly by the reagents used to preserve the milk. The "fresh" milk used by Van Slyke and Bosworth, from which the conclusion was drawn that the albumin is partly in colloidal and partly in true solution, was in reality milk to which 5 per cent chloroform had been added.

The data which we present in this paper show conclusively that the Pasteur-Chamberland filters which we used had a much finer porosity than those used by Van Slyke and Bosworth. We have decided to publish the data which we have already secured, since all our attempts to secure filters of any other make, which will fit the Briggs' apparatus, have been futile.

We have also made some study of another phase of Van Slyke and Bosworth's[8] filtration studies which we believe offers a partial explanation, at least, for the data from which they conclude that "fresh" milk has its lactalbumin partly in colloidal and partly in true solution. As already pointed out, the samples of milk which they regarded as "fresh" were preserved by the addition of 5 per cent chloroform. The quantitative estimations of lactalbumin in this milk and in the serum from this milk which passed through the filter were made by the heat-coagulation method. The point which we studied was the effect of allowing both whole milk and milk free from casein to stand in the presence of 5 per cent chloroform upon this method of determining lactalbumin.

The data which we offer on this phase of the question show very clearly that chloroform left in contact with either whole milk or diluted milk serum causes a marked reduction in the amount of albumin recoverable by heat coagulation when compared with the same milk analyzed fresh. In certain cases we found this difference alone to be sufficiently great to account for the apparent result secured by Van Slyke and Bosworth that a portion of the albumin is in true and a part in colloidal solution, assuming that the filters which they used allowed the albumin particles to pass into the filtrate.

[11] Ostwald, W., An introduction to theoretical and applied colloid chemistry, New York, 1917, 47.

EXPERIMENTAL.

All of our filtration studies were made using fresh milk from the University of Missouri dairy herd. Skim milk only was used, the fat being removed by running the whole milk at least twice through a closely skimming centrifugal separator at high speed and at a temperature of 43–45°C. In no case was it possible to detect the presence of fat in this milk by the Babcock test, using a skim milk test bottle.

We found it convenient in certain of our work to make comparative studies on the same milk at the same time. This was accomplished by connecting two of the Briggs' filters to the source of pressure by a T tube. A well charged cylinder of oxygen was used as the source of pressure. With a pressure gauge in the system and good connections we were able to maintain as much pressure as the apparatus would permit.

No attempt was made to recover the heat-coagulable portion of the protein in the filtrates which passed through the Pasteur-Chamberland tubes. Whenever it was desired to separate the nitrogen in the filtrates into protein and non-protein portions, this was accomplished by the addition of 20 cc. of Almen's tannic acid reagent to 10 cc. of the filtrate, after the addition of 90 cc. of water. The precipitates which formed were allowed to stand for 24 hours before being filtered off. The nitrogen was then determined separately in the precipitate and filtrate. In a number of cases no separation of protein from non-protein was made, the total nitrogen in the serum being determined directly on suitable portions. All nitrogen determinations were made by the Gunning modification of the Kjeldahl method, using copper sulfate as catalyser.

Van Slyke and Bosworth[8] assumed in their work that the serum passing through the Pasteur-Chamberland filters attained a constant composition after the first 50 to 75 cc. had passed through. We analyzed each of the successive 10 cc. portions of the filtrate from the first until at least 100 cc. of serum had been collected.

The Pasteur-Chamberland tubes which we used were obtained from the Central Scientific Company of Chicago.

Experiment 1. Comparison of Composition of Filtrate Using $CHCl_3$ *and* HCHO *as Preservatives.*—4 liters of fresh whole milk

were run through a centrifugal separator twice at 45°C., the crank being turned at a speed of 75 R.P.M. After cooling to 20°C., two portions of the skim milk of 1 liter each were carefully measured out. 50 cc. of the milk from one sample were replaced by an equal volume of chloroform, and 1.26 cc. of the other sample by an equal volume of 40 per cent formaldehyde solution. The two samples therefore contained, respectively, 5 per cent chloroform and 0.05 per cent formaldehyde. A third portion of the skim milk was subjected to immediate analysis for casein, soluble protein, and residual non-protein nitrogen, the so called soluble protein being precipitated from the casein filtrate by means of Almen's tannic acid reagent. The preserved portions of the milk were immediately placed in the filtering chambers of the Briggs' apparatus and a pressure of three to four atmospheres was applied continuously until 100 cc. of serum were obtained from the sample containing formaldehyde and 120 cc. from the sample containing chloroform. A little over 12 hours were required for the collection of the former, the latter requiring nearly 24 hours to pass through the filter.

The analysis of the fresh milk is shown in Table I, and of the successive portions of the serum from the two filters in Table II, the data representing the net results after deducting the necessary blanks for reagents, and calculating them to 100 cc. of milk.

The data presented in Tables I and II show that the Pasteur-Chamberland tubes which we used held back a great deal more of the nitrogenous constituents of the milk than those used by Van Slyke and Bosworth.[8] It is necessary to review only a portion of the data given by these authors to show how widely divergent our results are from theirs. For example, the lowest amount of "albumin" nitrogen which they recovered from the serum amounted to 0.0232 gm. per 100 cc. of milk, and one figure reported shows as much as 0.0578 gm. of "albumin" nitrogen obtained from the serum of a sample of milk to which 5 per cent chloroform had been added as preservative. As has already been pointed out, it is clear from an experimental point of view alone that the Pasteur-Chamberland filtering tube, or for that matter any other type of porous clay filter, is not the proper criterion by which to judge whether the nitrogenous constituents

of milk other than casein are in a state of molecular or colloidal dispersion. In our trials we apparently did not even succeed in

TABLE I.

Distribution of Nitrogen in 100 Cc. of Fresh Skim Milk, Experiment 1.

Constituent.	Nitrogen.		
	Portion 1.	Portion 2.	Average.
	gm.	gm.	gm.
Casein*	0.3886	0.3893	0.3900
Soluble protein†	0.1116	0.1138	0.1127
Non-protein	0.0424	0.0388	0.0406

* Casein determined by official method of Association of Official Agricultural Chemists.

† Protein precipitated from casein filtrate by Almen's reagent.

TABLE II.

Comparative Distribution of Nitrogen in 100 Cc. of Serum from Pasteur-Chamberland Filter, Experiment 1.

HCHO sample.				$CHCl_3$ sample.			
Sample No.	Protein N.	Non-protein N.	Total N.	Sample No.	Protein N.	Non-protein N.	Total N.
	gm.	gm.	gm.		gm.	gm.	gm.
1	—	—	0.0216	1	—	—	0.0269
2	0.0089	0.0262	0.0351	2	0.0080	0.0252	0.0336
3	—	—	0.0358	3	—	—	0.0276
4	—	—	0.0382	4	—	—	0.0269
5	0.0144	0.0268	0.0412	5	0.0007	0.0258	0.0265
6	—	—	0.0336	6	0.0033	0.0291	0.0324
7	—	0.0291	—	7†	—	—	0.0282
8	—	—	0.0375				
9	—	—	0.0398				
10	—	—	0.0415				
Average.....	0.0116	0.0270	0.0378*		0.0040	0.0269	0.0289

* Excepting Sample 1.

† Sample representing 70 to 120 cc. portion of filtrate.

recovering in the serum all the nitrogen of the milk which cannot be precipitated by tannic acid, about 35 per cent of it either

being adsorbed on the filter or existing in particles too large to pass through the pores.

Our data do, in a measure, substantiate one observation made by Van Slyke and Bosworth; namely, that more protein nitrogen passes through the filter when formaldehyde is used as a preservative than when chloroform is used. Calculation shows that only 3.5 per cent of the so called soluble proteins of the milk was recovered in the case of the chloroformed sample, while 10.3 per cent was recovered in the sample containing formaldehyde. The latter figure, however, is far short of the 92.21 per cent recovery reported by Van Slyke and Bosworth[8] for "albumin" in the case of milk containing formaldehyde. Data to be presented in a later experiment offer a probable explanation of the fact that chloroform retards the filtration of protein through the Pasteur-Chamberland filter. It will be shown there that chloroform when left in contact with lactalbumin solutions causes a partial precipitation of this protein.

Another interesting point emphasized by Van Slyke and Bosworth[12] as the result of their studies is that sour milk contains all the lactalbumin in true solution, their data showing that 100 per cent recovery of "albumin" is obtained from the serum of sour milk filtered through the Pasteur-Chamberland filter.

Experiment 2 was designed to test this point using our Pasteur-Chamberland filters.

Experiment 2. Comparison of Composition of Filtrates from Milk Containing No Preservative with Serum from Milk Whose Casein Has Been Removed by Lactic Acid.—Several liters of fresh milk were separated twice in a centrifugal separator at 45°C., the crank being turned at a speed of 75 R.P.M. 10 cc. of concentrated lactic acid were added to 1 liter of the skim milk. The casein was filtered off through cheese-cloth and the slightly cloudy filtrate filtered through a Pasteur-Chamberland tube under a pressure of three to four atmospheres. In this test the chamber of the Briggs' apparatus was surrounded by a large metal tub which was kept full of water at the temperature of

[12] Van Slyke, L. L., and Bosworth, A. W., *N. Y. Agric. Exp. Sta., Technical Bull. 48*, 1916; *J. Biol. Chem.*, 1916, xxiv, 191.

melting ice throughout the entire filtration, the outlet from the filter passing through a stopper in the bottom of the tub.

Separate analysis was made of the original milk, the nitrogen distribution being determined among the following constituents: casein, protein precipitable from the casein filtrate by Almen's tannic acid reagent, and non-protein nitrogen.

The filtration was relatively rapid in this test. Analyses were accordingly made of the first ten 10 cc. portions and of the next three 50 cc. portions, the last analysis thus representing the portion from 200 to 250 cc.

For comparison with the data secured in this test we offer the data secured from the filtration of a sample of skim milk, prepared at another time, the principal point of comparison being that it, too, was filtered at the temperature of melting ice, without the use of any preservative. No nitrogen distributions were made in this case, however, total nitrogen determinations only being made upon the first ten 10 cc. portions which passed through the filter. The casein and total non-casein nitrogen were determined in a separate portion of the milk. The data from this experiment are shown in Tables III and IV, Table III giving the analyses of the original samples of milk and Table IV the composition of the various portions of the serum.

In spite of the fact that the two samples of milk represented in this experiment were of entirely different composition and their filtration was carried out at different times, and also notwithstanding the fact that Sample II was merely the lactic acid whey, free from casein, there is a marked uniformity in the proportion of the non-casein nitrogen which passed through the filter in the case of the two samples. It is evident also that in both cases this consisted almost wholly of non-protein nitrogen and shows that our filters had a porosity of such fineness as to retain most of the proteins other than casein even after they had been rendered "soluble," as Van Slyke and Bosworth believe, by the lactic acid, and there was no casein present to adsorb them. We interpret this to mean that the albumin of sour milk is still in a colloidal condition and that it is not to be regarded as in true solution simply because a porcelain filter whose pores are of a certain degree of fineness will allow it to pass.

Experiment 3. Effect of Chloroform on Determination of Lactalbumin by Heat Coagulation.—The method most commonly used

TABLE III.

Distribution of Nitrogen in 100 Cc. of Original Milk, Experiment 2.

Constituent.	Sample No.	Nitrogen.		
		Portion 1.	Portion 2.	Average.
		gm.	gm.	gm.
Casein N	I*	0.4308	0.4321	0.4315
Non-casein N	I*	0.1557	0.1505	0.1531
Casein N	II†	0.4500	0.4494	0.4497
Protein N other than casein	II†	0.1007	0.1007	0.1007
Non-protein N	II†	0.0255	0.0268	0.0262
Total non-casein N	II†	—	—	0.1269

* Fresh skim milk filtered without any preservative at temperature of melting ice.

† Fresh skim milk, the lactic acid whey from which was filtered without preservative at temperature of melting ice.

TABLE IV.

Relative Distribution of Nitrogen in 100 Cc. of Serum from Pasteur-Chamberland Filters, Experiment 2.

Fresh milk.		Lactic acid whey.			
Sample No.	Total serum N.	Sample No.	Protein N.	Non-protein N.	Total N.
	gm.		gm.	gm.	gm.
1	0.0332	5	0.0038	0.0249	0.0287
2	0.0527	6	—	—	0.0323
3	0.0513	7	—	—	0.0336
4	0.0533	8	—	—	0.0352
6	0.0414	9	—	—	0.0340
7	0.0440	10	0.0064	0.0271	0.0335
9	0.0427	12	0.0064	0.0289	0.0353
10	0.0447	13	0.0064	0.0262	0.0326
Average*	0.0471	Average.	0.0056	0.0268	0.0331
Percentage of same nitrogen in original milk	30.7		5.5	100.0	26.0

* Excepting Sample 1.

for the quantitative estimation of lactalbumin in cow's milk is based on a coagulation of the casein filtrate by heat. The protein recovered by this method does not, however, represent all of the protein present in the casein filtrate. In every case, even after the most careful coagulation, it is possible to recover considerable protein from the "albumin" filtrate by tannic acid, and, in the senior author's experience this fraction is frequently greater than that thrown down by heat coagulation. Even this procedure leaves a small quantity of nitrogen which is evidently not of true protein nature, since it fails to respond to the most delicate tests for protein.

It has long been a disputed point whether the non-coagulable protein of milk serum is to be regarded as of a proteose-peptone character. It is not our purpose to review this controversy at this time, but it may be pointed out that there never has been any conclusive proof offered that fresh milk contains these secondary proteins. It is the author's opinion that much of this controversy has arisen because of the failure to appreciate the fact that heat coagulation seldom if ever gives a quantitative measure of the so called soluble animal proteins. This is particularly applicable to the albumin and globulin in milk. This has not been properly appreciated by dairy chemists in recent years, notwithstanding the fact that foreign investigators have been emphasizing it for nearly 50 years.

Zahn,[13] as long ago as 1870, pointed out that heat does not give a complete precipitation of albumin from milk. Hofmeister,[14] applying this method to animal fluids in general, found that it rarely leaves the filtrate free from protein. Neumeister,[15] studying the question of the presence of peptones in milk, concluded that heat coagulation in weakly acid solutions always results in the formation of primary proteoses from the coagulated proteins, which he regards as especially easily hydrolyzed. Sebelien[16] found in his extensive investigation of milk proteins that a large part of the albumin of milk remained uncoagulated at the boiling temperature, as high as one-fourth to one-third of the total albumin being lost. Schloss-

[13] Zahn, F. W., *Arch. ges. Physiol.*, 1870, iii, 76.
[14] Hofmeister, F., *Z. physiol. Chem.*, 1878–79, ii, 288.
[15] Neumeister, R., *Z. Biol.*, 1888, xxiv, 272.
[16] Sebelien, J., *Z. physiol. Chem.*, 1889, xiii, 135.

mann,[17] reviewing the various methods of estimating milk proteins, also points out that it is impossible to precipitate completely the albumin by heat.

These statements, which are by no means claimed to be a complete bibliography on the subject, only serve to confirm the author's own conviction based upon a wide experience and much study of the question of the quantitative determination of the lactalbumin in cow's milk. The writer hopes to publish some of his own experimental work on this question in the near future.

TABLE V.

Distribution of Protein in 100 Gm. of Milk when Fresh and after Standing 7 Days with 5 Per Cent $CHCl_3$.

Sample No.	Constituent.	Fresh skim milk.	Skim milk after 7 days with $CHCl_3$.	Variation from original.	Casein filtrate after 7 days with $CHCl_3$.	Variation from original.
		gm.	*gm.*	*per cent*	*gm.*	*per cent*
I	Heat-coagulable protein	0.315	0.239	−40.0	—	—
I	Residual protein	0.322	0.328	+1.7	—	—
II	Casein	2.932	2.889	−1.4	—	—
II	Heat-coagulable protein	0.405	0.370	−8.6	—	—
III	Casein	3.016	2.800	−7.1		
III	Heat-coagulable protein	0.442	0.299	−32.4	0.074*	−13.8
					0.297	
III	Residual protein	0.301	0.417	+38.5	0.384	+27.6
III	Non-protein nitrogen	0.030	0.055	+83.3	0.044	+46.7

* Precipitate which formed on standing.

In their studies on the filtration of "fresh" milk through the Pasteur-Chamberland filter, Van Slyke and Bosworth[8] determined the albumin in both whole milk and milk serum by coagulation with heat. Not only does this method leave a large part of the albumin in the filtrate, but the amount of albumin which can be recovered by this method is likely to be still less because of the presence of chloroform in the milk, which was the preservative used for their "fresh" milk studies.

The data presented in Table V show the results of several experiments in which the heat-coagulable proteins were determined

[17] Schlossmann, A., *Z. physiol. Chem.*, 1896–97, xxii, 197.

in samples of fresh, unpreserved milk, and again in the same milk after standing for several days in the presence of 5 per cent chloroform. In the case of Sample III, additional information on this point was secured by precipitating the casein from a separate portion, making the filtrate and washings up to a volume of 200 cc., adding 10 cc. of chloroform, and completing the analysis after standing for several days at room temperature. The method used for coagulation of the albumin was to neutralize the casein filtrate with NaOH solution, using phenolphthalein as indicator, add 0.3 cc. of 10 per cent acetic acid solution, bring the solution rapidly to the boiling point, and boil gently for 10 minutes. The coagulum was then filtered off and washed, and the nitrogen determined, as in all other cases, by the Kjeldahl-Gunning method. The data presented are the average of duplicate determinations.

Columns 5 and 7, Table V, show the percentage variation of the second determinations from the original analyses. The point which it is desired to emphasize is the loss in heat-coagulable protein in each case, ranging from nearly 10 per cent in Sample II to 40 per cent in Sample I. An interesting point which developed in Sample III in connection with the casein filtrates which were allowed to stand in contact with chloroform was the gradual formation of a white precipitate. This was filtered off and its nitrogen determined separately. The duplicate determinations of this precipitate were, respectively, 0.080 and 0.068 gm. of protein. When considered with the protein recovered by heat coagulation, the loss from the original analysis was 13.8 per cent, but the portion recovered by heat alone was practically identical with that recovered from the skim milk in contact with the chloroform.

The data seem to be very suggestive of the probable cause of the major part of the loss in heat-coagulable protein when milk is preserved with chloroform. They also offer a very plausible explanation of the apparent result secured by Van Slyke and Bosworth[8] that chloroformed milk allows much less heat-coagulable protein to filter through the Pasteur-Chamberland filters than milk containing formaldehyde. We have so far been unable to find any theoretical grounds to support the idea that chloroformed

milk represents more nearly the natural condition, so far as the state of the protein is concerned, than milk to which formaldehyde had been added in amount sufficient merely to prevent bacterial development. Our own experience is also contrary to this idea.

The authors do not consider it likely that proteolysis was a factor in causing the results indicated in Table V.

SUMMARY AND CONCLUSIONS.

Experiments are reported in which fresh skim milk, skim milk preserved with 5 per cent chloroform, skim milk preserved with 0.05 per cent formaldehyde, and the lactic acid whey from fresh skim milk were filtered through Pasteur-Chamberland filtering tubes under pressure. The total protein passing through the filters was determined in each case by precipitation with Almen's tannic acid reagent, and the non-protein nitrogen in the filtrate from the precipitate thus formed.

The amount of non-casein protein recovered in the filtrate did not in any case exceed 10 per cent of the non-casein protein in the original milk, and in most cases the amount recovered was considerably less than this figure. There was also only a partial recovery of the non-protein nitrogen of the original milk in the experiments with chloroformed and formaldehyde-treated milk. These results are widely divergent from those reported by previous investigators.

The variation in the size of the pores of different Pasteur-Chamberland filters which is indicated by a comparison of our data with those secured by others who have filtered milk through these filters shows conclusively the fallacy of drawing conclusions regarding the true state of solution of non-casein proteins of milk based on filtration studies of this character.

Some remarks are offered regarding the fallible practice of determining the "albumin" of milk by heat coagulation. Several experiments are reported showing how chloroform left in contact with milk greatly depresses the amount of protein which can be recovered from casein filtrates by this method. One experiment is reported indicating that this depression may be due partially

to a precipitation of heat-coagulable protein by the chloroform. A plausible explanation is thereby offered for the results secured by other investigators who find that chloroformed milk apparently allows less heat-coagulable protein to pass through the Pasteur-Chamberland filter than milk preserved with formaldehyde, or sour milk.

1st Lieutenant Robert G. Scott, aerial observer, was killed in action on October 4, 1918.

Endowed with a brilliant mind, a splendid physique, and a tireless energy, his keen interest in biochemistry gave rich promise of a splendid future. Courageous and dauntless to a fault, his country's call naturally led him into the field of service which he chose. Possessed with a happy disposition, he greatly endeared himself to his associates who mourn his loss to science at the threshold of his career.

THE STATE OF PROTEINS IN COW'S MILK.

By L. L. VAN SLYKE and A. W. BOSWORTH.

(Received for publication, October 21, 1918.)

In the preceding article Palmer and Scott have drawn certain conclusions concerning the condition of albumin in cow's milk, based upon the separation of serum from milk by a method outlined by us in a previous paper.[1] This method involves filtration through porous clay filters. In our paper we stated:

"It has been found by Rupp that the filter appears to have the power of adsorbing some of the constituents of the serum until a volume of 50 to 75 cc. has passed through, after which the filtered serum is constant in composition. In our work, therefore, the first portion of serum filtered is not used."

We did not "assume that the serum passing through the Pasteur-Chamberland filter attained a constant composition after the first 50 to 75 cc. had passed through," but actually found this to be the case, giving credit to Rupp for the original observation, however. That this is the case is also shown by the figures given by Palmer and Scott in their Table II. In the case of the milk treated with formaldehyde the total nitrogen in the serum of the first ten 10 cc. portions varied from 0.0216 to 0.0415 gm. As they used much finer filters than we did it would require the passage through them of a greater volume of liquid in order that they might become saturated with respect to those substances adsorbed. Had they discarded the first 100 cc. or so and then collected a total of 250 cc. or more from which to draw samples for analysis the results obtained by them would have more weight when used to criticize the results secured by us.

With respect to their work on chloroformed milk we can only say that the action of chloroform upon the proteins of milk is a

[1] Van Slyke, L. L., and Bosworth, A. W., *J. Biol. Chem.*, 1915, xx, 135.

progressive one, due to two or more factors, one of which is the action of the hydrochloric acid produced. After a few weeks the proteins may be completely precipitated from the milk. The changes noted by them at the end of 7 days are therefore not comparable to the changes which might have taken place in our filtrations lasting from 12 to 36 hours.

THE DIETARY PROPERTIES OF THE PEA (VICIA SATIVA).*†

By E. V. McCOLLUM, N. SIMMONDS, and H. T. PARSONS.

(From the Laboratory of Chemical Hygiene, School of Hygiene and Public Health, Johns Hopkins University, Baltimore.)

(Received for publication, December 19, 1918.)

The pea and the navy bean have become better established as staple human foodstuffs in the United States than have any other of the legume seeds, but in recent times the cow-pea, soy bean, and peanut have been promoted. The soy bean is second only to rice in importance as a foodstuff in the Orient. Several studies have been made of the nutritive values of certain of the isolated proteins from these seeds by Osborne and Mendel (1), and to some extent the peanut and soy bean have been investigated in their dietary properties by Osborne and Mendel (2), Daniels and Nichols (2), and Daniels and Loughlin (2). These authors have attributed unusually high values to both of these seeds, especially with respect to the quality of their proteins and the content of fat-soluble A. From their data one would conclude that they are dietetically unique among seeds in these respects.

For several years we have been engaged in the study of the special dietary properties of our more important foodstuffs, with a view to discovering the exact nature of their deficiencies from the dietary standpoint. These studies have now included all the more important cereal grains (3), wheat germ (4), the navy bean (5), certain leaves (6), mixtures of cereal grains and the legume seeds (7), and complex mixtures of seeds including both the cereal

* A part of the experimental work reported in this paper was carried out by the authors at the Wisconsin Experiment Station.

† We employed split peas which were soaked in distilled water and heated 75 minutes in an autoclave at 15 pounds pressure; they were then dried in a current of air about 70°C., and ground.

grains and the legume seeds, especially the pea and bean (8). The results of these studies have led us to formulate a new classification of the foodstuffs based on their biological functions (9). Among the vegetable foods, those which are functionally storage tissues as the seeds, tubers, certain roots, and to some extent certain modified leaves, as the thick leaf of the cabbage, show decidedly greater dietary deficiencies than do those which are the seat of great metabolic activity. Examples of the latter class are the leaves generally, but more especially the thin leaves which are not filled with reserve food materials, the germ of the seed, and probably also those regions of the tubers and fleshy roots which are rich in cellular elements. The seed, tuber, and root group are all decidedly deficient in calcium, sodium, and chlorine among the inorganic elements, and in the quality of their proteins, and with few exceptions in the content of fat-soluble A. The leafy portions of the plants approximate complete foods, and in some plants are actually complete foods which suffice for years to maintain a state of good nutrition in animals. An example in point is the prairie grass on which the bison subsisted throughout the year. It is true they had access to salt licks and manifested a distinct craving for salt. There seems on the other hand to be no mammal which is able to subsist entirely upon a diet of seeds, but certain birds when fed a supplementary supply of calcium in water, shells, grits, etc., appear to be satisfactorily nourished on such a restricted diet. It is interesting to note that millet seed, which among the seeds is especially rich in fat-soluble A, has found favor with bird fanciers as a regular constituent of rations.

The present paper contains a report of the dietary properties of the common pea. The data are recorded because of their interest in contributing to the evidence in support of the general theory which we have formulated; *viz.*, that vegetable foods having similar functions have likewise similar dietary properties (10).

We also present records of experiments which show the supplementary relationships between the proteins of the pea and casein, gelatin, zein, and lactalbumin, respectively. It is of great interest that while both casein and zein supplement the deficiencies of the pea proteins, gelatin and lactalbumin do not. From the failure of lactalbumin to supplement the proteins of the pea, or to induce growth when fed in the amounts used in the experi-

ments described in this paper, we have come to the tentative conclusion that lactalbumin is either an incomplete protein or a poorly constituted one. If lactalbumin were actually so good a protein as the experiments of Osborne and Mendel indicate, the 9 per cent with which we supplemented a content of 10 per cent of pea protein in Lot 767, Chart 5, should itself have been sufficient to induce good growth. We have elsewhere shown that 8 per cent of protein derived from the mixture of proteins contained in milk, is sufficient to lead to nearly normal growth (11), and 9 per cent of a protein mixture, two-thirds of which is derived from rye and the remainder from flaxseed oil meal, is of such good quality that with this amount in the diet young rats grow at the optimum rate to full adult size (12).

We have been unable to obtain any growth in young rats with diets containing 18 per cent of lactalbumin as the sole source of protein (8), when the remainder of the food mixture was so constituted that good growth could be secured when the protein (18 per cent) of the diet was casein. We are forced to the conclusion that lactalbumin is a poorly constituted or an incomplete protein, and that the excellent results of Osborne and Mendel were due to the high proportion of nitrogen derived from "protein-free milk" which was present in their food mixtures and served to supplement the lactalbumin with respect to some as yet undetermined cleavage product which is essential for growth (13).

Zein is lacking in glycocoll and tryptophane and contains little, if any lysine, and is very low in cystine, if indeed this amino-acid is present. Since the proteins of the pea are fairly well supplemented by zein, it follows that the limiting amino-acid in the pea is neither tryptophane, lysine, nor cystine (see Chart 8). Glycocoll has been shown by McCollum and Hoagland (14) to be readily synthesized by the mammalian organism.

Chart 1.—Lot 866 illustrates the slow rate of growth with early stunting, of young rats which were fed peas sufficient to make about 20 per cent of protein, supplemented with a salt mixture[1] and fat-soluble A (in butter fat). A comparison with Lots 976, 903, and 1030 (Charts 1 and 2) shows that the peas were supple-

[1] For composition of salt mixture 185 see McCollum, E. V., and Simmonds, N., *J. Biol. Chem.*, 1918, xxxii, 191.

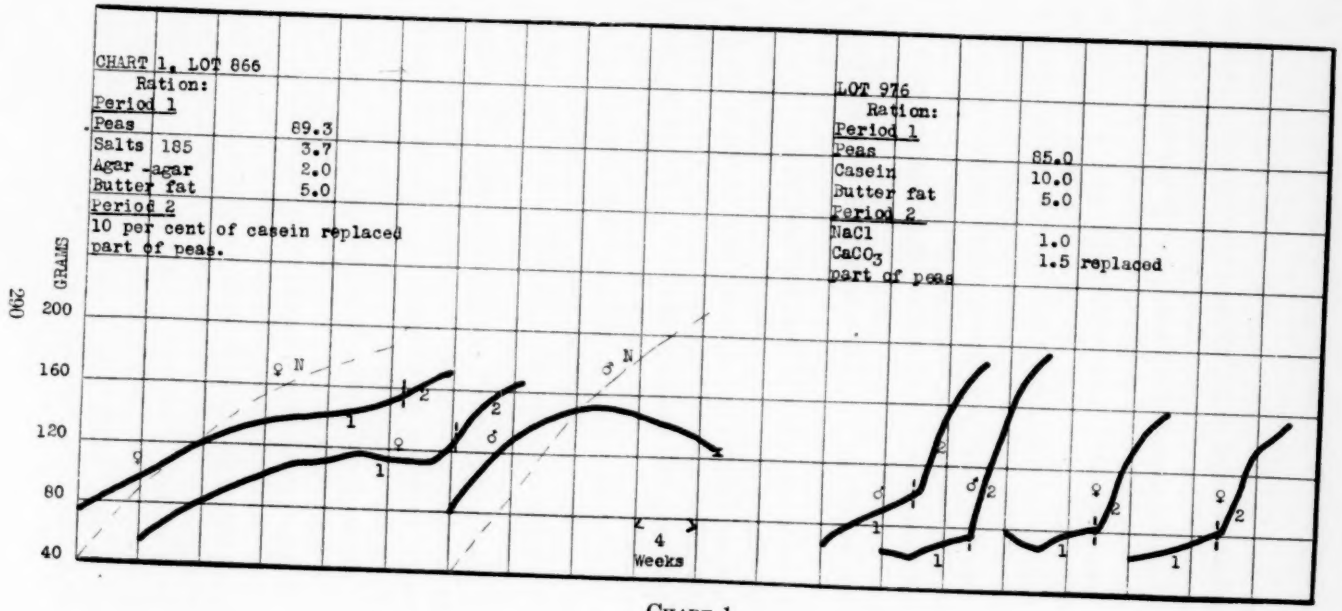
CHART 1. LOT 866
Ration:
Period 1
Peas 89.3
Salts 185 3.7
Agar-agar 2.0
Butter fat 5.0
Period 2
10 per cent of casein replaced part of peas.
LOT 976
Ration:
Period 1
Peas 85.0
Casein 10.0
Butter fat 5.0
Period 2
NaCl 1.0
CaCO3 1.5 replaced part of peas
GRAMS
200
160
120
80
40
4 Weeks

CHART 1.

mented with respect to all necessary factors except protein. In Period 2, the addition of casein led to a sharp response with growth in one rat, and a distinct acceleration of growth in another. This shows that the pea proteins are of very poor quality when fed as the sole source of nitrogen. The rate of growth on this diet containing 20 per cent of pea protein is not so good as has been observed with diets containing but half as much protein derived from one of the cereal grains (3). That the addition of a salt mixture is necessary is shown by the records of Lot 976, Chart 1. The pea is fairly rich in the fat-soluble A as is shown by Chart 2, Lot 1030.

Lot 976, Period 1, shows that even when supplemented with protein and fat-soluble A, peas to the extent of 85 per cent of the food mixture cannot support growth, because of the shortage of certain inorganic elements. Period 2, shows that no inorganic additions other than calcium, sodium, and chlorine are necessary in order to make the diet satisfactory for the support of growth at the optimum rate. We have not, up to the present time, found any seed or mixture of seeds which did not require inorganic salt additions before growth could take place.

Chart 2.—Lot 903, Period 1, illustrates as did Chart 1, Lot 866, that the proteins of the pea are not of very good quality for the support of growth. 45 per cent of peas furnishes a protein content of about 10 per cent of the food mixture, but the animals were not able to grow on this diet even though the peas were supplemented with respect to all other factors. This amount of protein from one of the cereal grains would suffice for the support of growth at a better rate (3). In Period 2, when 9 per cent of casein was added, there was a prompt response with growth in all animals. Peas are not so high in hemicelluloses as are navy beans, and fermentation of indigestible carbohydrates is not so depressing a factor as in diets containing a high content of navy beans. If the proteins of the pea were of good quality it should be apparent from the behavior of animals fed the types of diets which we have employed.

Lot 1030 illustrates the remarkable growth and good reproduction records of rats which were restricted from weaning to a diet of peas supplemented with casein, calcium carbonate, and sodium chloride. One female had four litters of young, but none

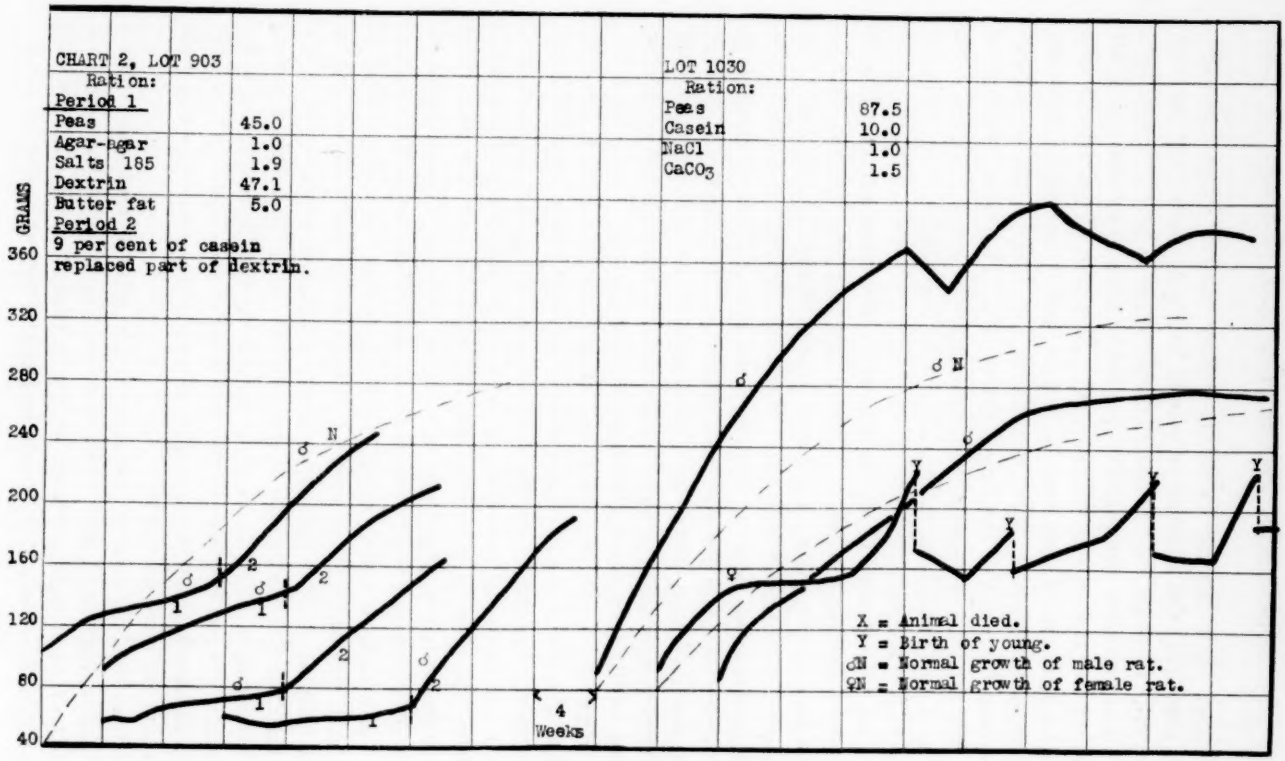
CHART 2, LOT 903
Ration:
Period 1
Peas 45.0
Agar-agar 1.0
Salts 185 1.9
Dextrin 47.1
Butter fat 5.0
Period 2
9 per cent of casein replaced part of dextrin.
LOT 1030
Ration:
Peas 87.5
Casein 10.0
NaCl 1.0
CaCO3 1.5
GRAMS
360
320
280
240
200
160
120
80
40
4 Weeks
X = Animal died.
Y = Birth of young.
♂N = Normal growth of male rat.
♀N = Normal growth of female rat.

CHART 2.

of them were reared. The other at the age of about 10 months had her first and only litter of young, but these died when 15 days old. This diet would have been improved by the addition of butter fat (fat-soluble A) (see Chart 7, Lot 757). It is interesting to observe that without addition of the latter factor, such good growth and reproductive capacity could be secured. The results of this experiment demonstrate that there can be no very serious injury resulting from the feeding of a liberal amount of peas over a long period of time. In this respect peas appear to be superior to the navy bean which seems to cause injury when fed in liberal amounts to growing rats (5). Lathyrism has, so far as we are aware, not been observed with the species of pea we have employed in our experiments, but only with *Lathyrus sativus* (15) which belongs to the sweet pea family.

Chart 3.—Lot 754 illustrates well the great importance of adequately supplementing a diet in which one seed furnishes the major portion of the diet, with salts to supply suitable inorganic additions and with protein. This food mixture was similar to that of Lot 1030, Chart 2, except that it contained butter fat, and a complex salt mixture, instead of a simple addition of calcium carbonate and sodium chloride. This was one of our earlier experiments and the salt mixture was such as to furnish qualitatively all the elements except iodine which are needed by a growing animal, but the elements calcium, sodium, and chlorine were not furnished in such liberal amounts as in Lot 1030 or as to promote optimum nutrition. This was apparently the cause of the failure of Lot 754 to grow as well as the former.

We wish to emphasize that peas supplemented with respect to protein, fat-soluble A, and with a complex salt mixture, as described in Lot 754, Chart 3, failed to be satisfactorily nourished. Lot 757, Chart 7, which contained but half as much peas and was supplemented with essentially the same purified additions, was much better nourished as shown by both growth curves and reproduction records. In the latter group the number of young produced may be regarded as normal since two females produced nine litters (thirty-eight young), but none were successfully weaned. Such results strongly suggest the presence in peas of some substance or substances which prove injurious when taken in large amounts. It seems, however, that the toxicity of peas, if there

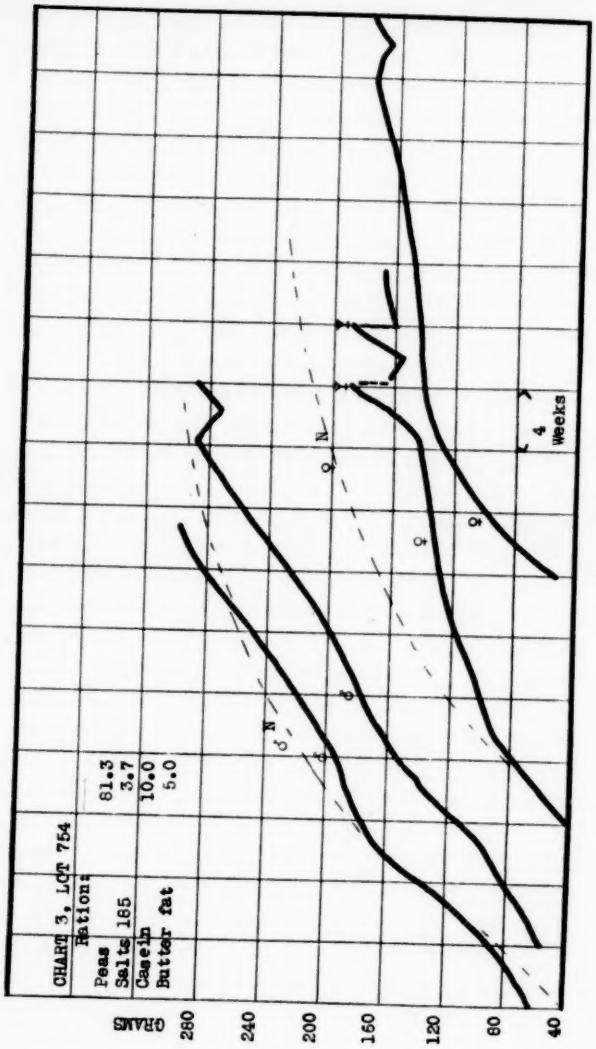
CHART 3, LOT 754
Ration:
Peas 61.3
Salts 185 3.7
Casein 10.0
Butter fat 5.0
GRAMS
280
240
200
160
120
80
40
4 weeks

CHART 3.

be any, is but slight and only manifests itself when diets extremely rich in peas are persisted in over a long period. In the quantities in which peas ever enter into human dietaries there can be no danger of the development of such a syndrome as lathyrism, but there may be a depression of function when the diet is distinctly unsatisfactory in some other factor.

Chart 4.—Lot 627 was fed a diet consisting of a purified food mixture supplemented with butter fat (fat-soluble A) and 5 per cent of peas. The latter was the sole source of the dietary factor water-soluble B. The animals were able to grow for a month, after which they failed rapidly. It is evident that this amount of peas is entirely insufficient to furnish the antineuritic factor, for two rats suffered from polyneuritis after 8 and 10 weeks respectively. Even so small an amount of wheat germ as 2 per cent of the food mixture suffices for the support of growth at nearly the normal rate when the diet is otherwise composed of purified foodstuffs and butter fat to furnish fat-soluble A (16). The cell-rich tissues of the plant such as the germ are better sources of the water-soluble B than are the entire seeds, but these in turn are better than is the endosperm.

Lot 695 was fed a diet which contained 25 per cent of cooked, dried peas, and consisted otherwise of purified foodstuffs and butter fat to furnish the fat-soluble A. Although the peas had been cooked under pressure of 15 pounds for $1\frac{1}{4}$ hours and dried in a current of hot air, one-fourth of the food mixture derived from peas so treated sufficed to supply enough of the water-soluble B to enable young rats to grow to the full adult size at the normal rate. Further studies are in progress to determine the minimum amounts of each of the more important seeds which meet the requirements of the growing young for this dietary factor. For the pea, it lies somewhere between 5 and 25 per cent of the food mixture (8). The amount of water-soluble B which is sufficient for growth is not necessarily enough for successful rearing of young. The demands of the nursing mother for this factor appear to be much greater than for the young for growth after the weaning period (17).

Chart 5.—Lot 767 presents curves of growth which are of remarkable interest in that they show that the proteins of the pea are not supplemented by lactalbumin. This protein has been

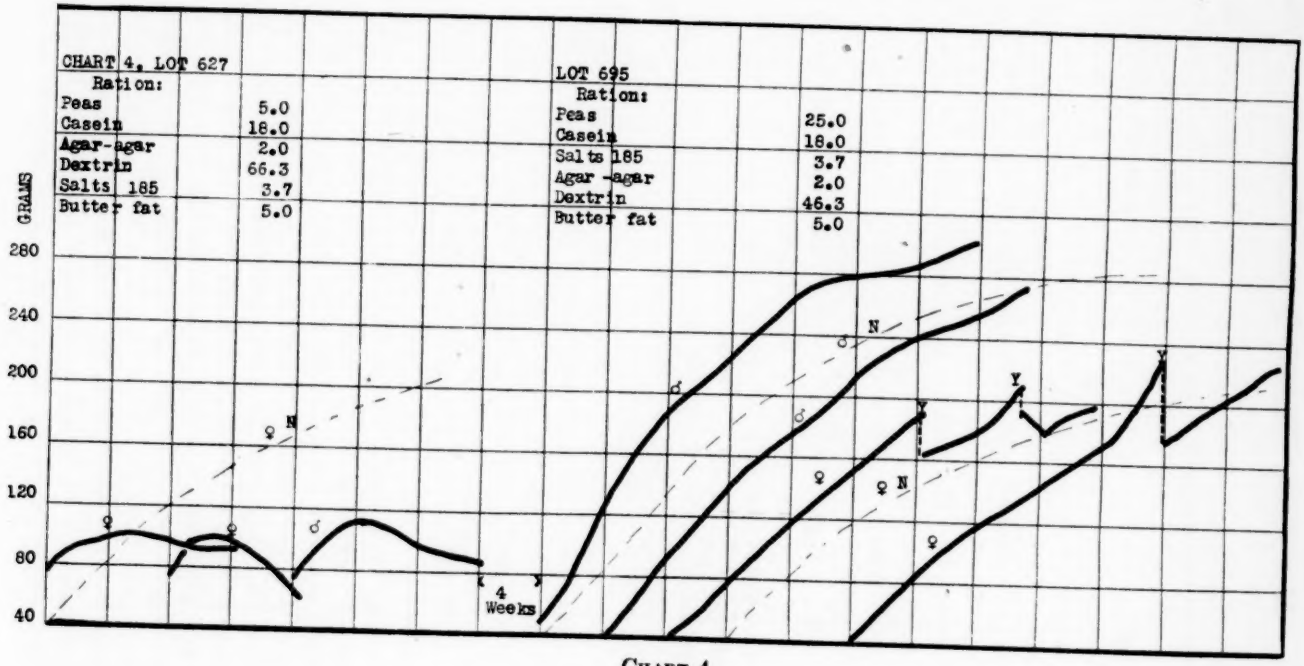
CHART 4, LOT 627
Ration:
Peas 5.0
Casein 18.0
Agar-agar 2.0
Dextrin 66.3
Salts 185 3.7
Butter fat 5.0
LOT 695
Ration:
Peas 25.0
Casein 18.0
Salts 185 3.7
Agar-agar 2.0
Dextrin 46.3
Butter fat 5.0
GRAMS
280
240
200
160
120
80
40
4 Weeks
N
Y

CHART 4.

said by Osborne and Mendel to be of greater biological value than any of the other purified proteins studied (13). If this is true we must accept the view that it is highly satisfactorily constituted with respect to its content of all the essential amino-acids and can be converted into tissue proteins to an extent nearer quantitative than can any other protein thus far studied. If this were true, 9 per cent of lactalbumin should be capable of supporting growth at a rate approximately as great as is possible in this species.

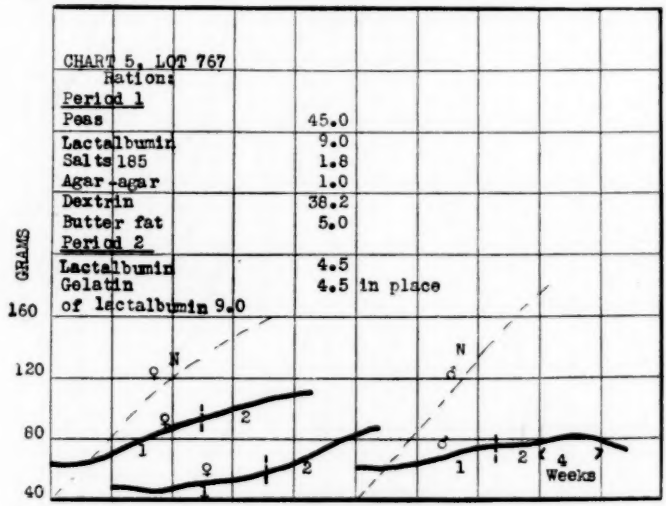

CHART 5.

Chart 6.—Lot 756 shows gelatin, like lactalbumin, to be unsuited for making good the deficiencies of the pea proteins. In Period 1 the diet was similar in all respects to that of Lot 757, Chart 7, except that gelatin replaced casein, in like amount. The diet containing the casein together with pea protein induced growth, whereas that containing gelatin and pea proteins did not. In Period 2, the diet was made up with 9 per cent of casein replacing the gelatin. There was an immediate response with growth in all the animals.

Chart 7.—Lot 757. In marked contrast to lactalbumin and gelatin, casein is a good protein for supplementing the deficiencies of the proteins of the pea. The diet of this group was similar in

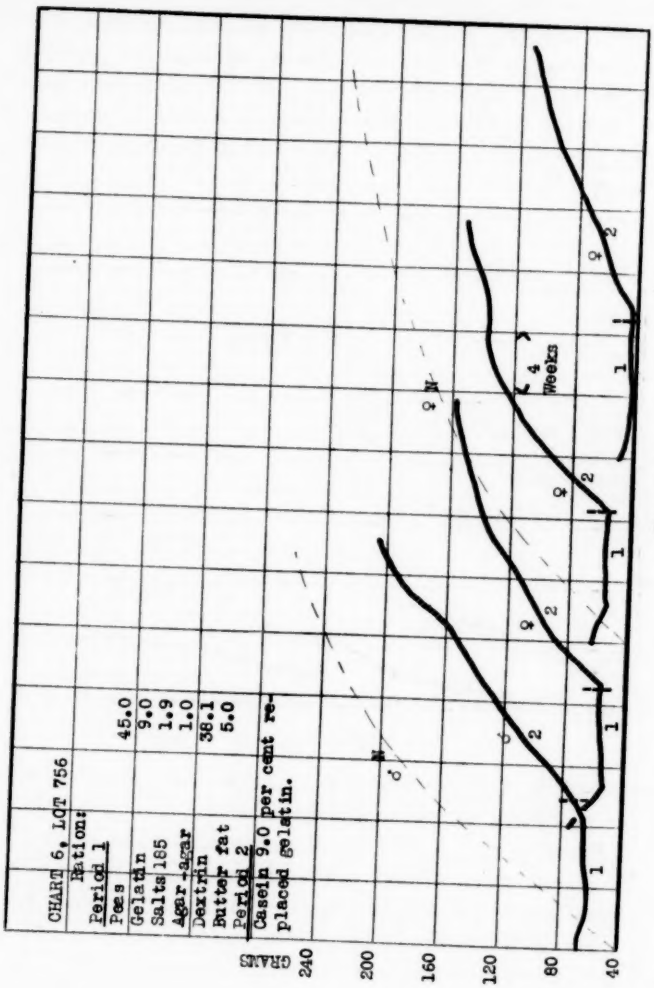
CHART 6, LOT 756
Ration:
Period 1
Peas 45.0
Gelatin 9.0
Salts 185 1.9
Agar-agar 1.0
Dextrin 38.1
Butter fat 5.0
Period 2
Casein 9.0 per cent replaced gelatin.
GRAMS
240
200
160
120
80
40
♂ N
♀ N
♂ 2
♀ 2
♂ 1
♀ 1
4 Weeks

CHART 6.

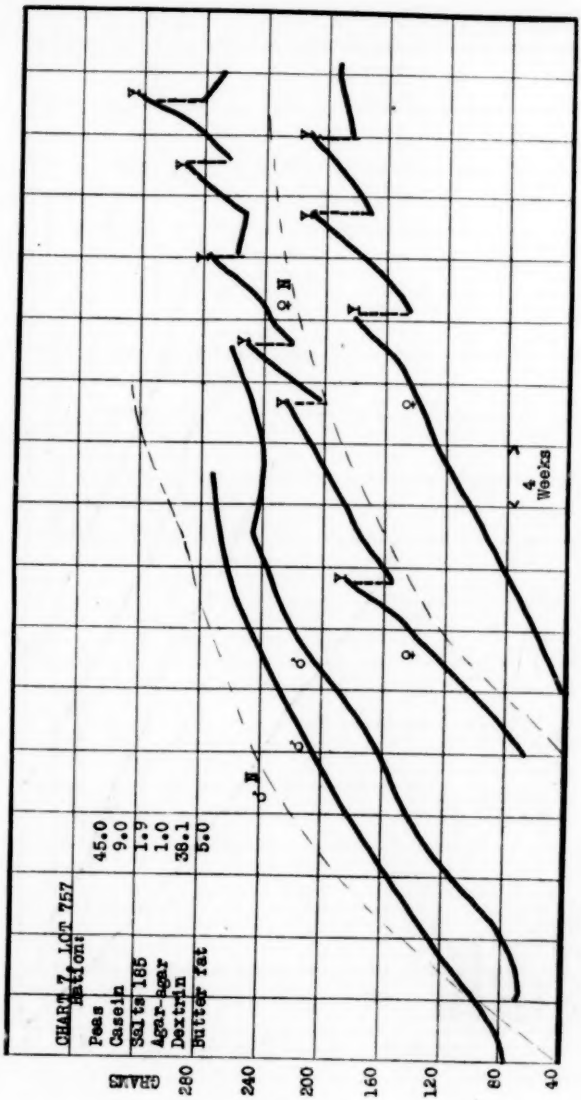
CHART 7. LOT 757
Ration
Peas 45.0
Casein 9.0
Salts 185 1.9
Agar-agar 1.0
Dextrin 38.1
Butter fat 5.0
GRAMS
280
240
200
160
120
80
40
4 Weeks

CHART 7.

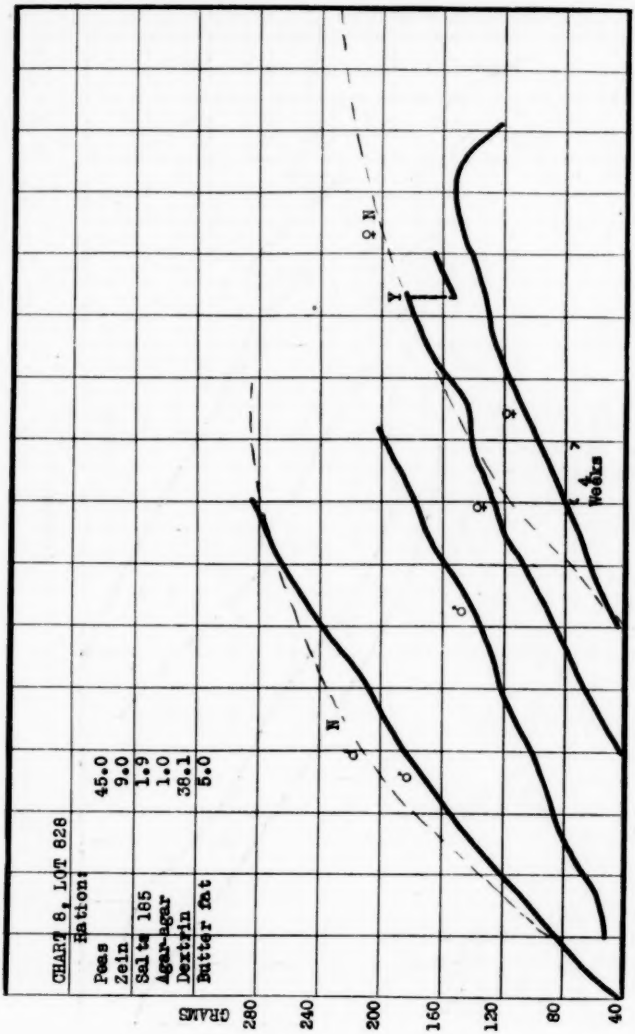
CHART 8, LOT 628
Ration:
Peas 45.0
Zein 9.0
Salts 165 1.9
Agar-agar 1.0
Dextrin 38.1
Butter fat 5.0
GRAMS
280
240
200
160
120
80
40
N
♂
♀
♂
♀
♂
♀

every respect to that of Lot 767, Chart 5, except that in the latter lactalbumin replaced casein. This supports the view that lactalbumin is a poorly constituted protein.

Chart 8.—Lot 828 makes it clear that zein is a moderately good supplementary protein for the proteins of the pea. The value of the mixture of approximately equal parts of zein and pea proteins is apparently distinctly greater than half that of an equal amount of casein, for about 15 per cent of the latter is required when the diet is otherwise satisfactory to promote growth at about the normal rate (18).

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MODIFICATIONS OF BENEDICT'S AND FOLIN'S QUANTITATIVE SUGAR METHODS.

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(Received for publication, December 16, 1918.)

Benedict's method[1] has proved satisfactory for estimation of sugar in urine when the concentration is above 0.2 per cent. Folin's[2] modification of Benedict's method is especially useful for micro-titration of urine containing much sugar. Since both these methods require potassium thiocyanate, which unfortunately has been unobtainable for some time, the writer substituted for it sodium thiocyanate which is easily secured.

Benedict's solution was prepared using 105 gm. of NaCNS (equivalent to 125 gm. of KCNS) and proved to be a perfect substitute for the usual reagent. The titrations with urines were identical with the regular reagent and with the substitute. In preparing the reagent we dissolve the sodium citrate first in 650 cc. of hot water, then the (monohydrated) sodium carbonate, and finally the sodium thiocyanate. After filtering the solution through cloth, the copper sulfate solution is added without waiting for it to cool.

On substituting NaCNS for KCNS in Folin's mixture of salts titration figures were obtained that were distinctly too low. The same result was secured when the thiocyanates were weighed out separately (from the phosphate and carbonate) for each estimation, thus eliminating the possibility of the error being due to a lack of uniformity in the mixing of the salts.

After experimenting it was found that correct titrations could be obtained by reducing the amount of sodium thiocyanate somewhat, and using periods of boiling half as long as those di-

[1] Benedict, S. R., *J. Am. Med. Assn.*, 1911, lvii, 1193.

[2] Folin, O., and McEllroy, W. S., *J. Biol. Chem.*, 1918, xxxiii, 513.

rected by Folin (0.5, 1.0, and 1.5 minutes instead of 1, 2, and 3). The results were as close to those secured by Folin's technique as duplicate estimations by the latter method; in other words, practically identical. The comparison was made with several different concentrations of sugar (urines) between 0.5 and 5.5 per cent. Both with slow titration (six to eight portions of urine added) and with rapid titration (three portions) the results were satisfactory.

For each estimation we use 4 gm. of a salt mixture containing 20 gm. of sodium thiocyanate, 60 gm. of dry sodium carbonate, and 100 gm. of disodium phosphate. We have found it convenient to use a small flask for the analysis instead of a test-tube.

THE INCREASE IN NITROGEN METABOLISM OF THE DOG, FOLLOWING THE ADMINISTRATION OF DESICCATED THYROID GLAND.

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(Received for publication, January 2, 1918.)

INTRODUCTION.

The following experiments were undertaken to determine how serviceable are small doses of commercial desiccated thyroid gland administered by mouth, as a means of increasing nitrogen elimination in the dog. That thyroid gland preparations cause an increase in nitrogen elimination in dogs has been reported in carefully balanced feeding experiments (1–10). The application of these findings to experimental work is so frequent as to justify additional study of this subject in the hope of making the procedure more practicable. We have undertaken to follow the nitrogen excretion on dogs which received only sugar solutions, thus eliminating the determination of a nitrogen intake, and to obtain figures for a dosage which would produce a marked increase in the nitrogen elimination.

EXPERIMENTAL.

The following general procedure was carried out for each experiment. A healthy, well nourished animal was kept for 24 to 48 hours on water, then each day within ½ to 1 hour of the same time the bladder urine was removed by catheter, the bladder washed free of urine, the animal weighed, and a measured amount of sugar water given by stomach tube. The sugar solutions contained a mixture of 125 gm. of cane sugar and 62 gm. of glucose to 1,000 cc. of water. Thyroid or dried veal pills when administered were placed in the throat before feeding the sugar water by stomach tube. A mixture of equal parts of desiccated thyroid preparation from Armour and Company, and from Parke, Davis and Company was used. This preparation contained 10 per cent

TABLE I.

Summary of Literature on Thyroid Preparations Administered to the Dog by Mouth.

Experimenter.	Experiment No.	Weight of dog.	Substance.	Dose.	Food.	Average daily N balance.	N balance day of administration.	Average increase in N in urine.	Increase in N on day of administration.	Days of administration.	Remarks.
		kg.		*gm.*			*gm.*	*per cent*	*per cent*		
Roos (1)	1	8.5	Dried and powdered thyroid.	3 (1 dose)	Dog biscuit, milk.		−0.60		21	1	*
	2	7.9	" " "	6 (1 ")	" " "		−0.82		24	1	*
Roos (2)	3	12.1	Dried thyroid.	5 (1 ")	" " "		−0.36		4	1	Low iodine.
	4	11.9	" "	5 (1 ")	" " "		−1.40		16	1	High "
	5	12.1	" "	5 (1 ")	" " "		−1.25		13	1	Low " *
	6	12.6	" "	5 (1 ")	" " "		−0.94		10	1	High "
	7	13.0	" "	5 (1 ")	" " "		−1.22		13	1	Low "
	8	13.0	" "	5 (1 ")	" " "		−1.48		16	1	High "
	9	13.7	" "	5 (1 ")	" " "		−0.18		2	1	No "
	10	13.9	" "	5 (1 ")	" " "		−1.10		0	1	" " *
	11	14.4	" "	5 (1 ")	" " "		−0.95		10	1	High "
Roos (3)	12	10.2	Thyroiodine.	0.05 (1 ")	" " "		−1.28		30	1	*
	13	10.1	"	0.06 (1 ")	" " "		+0.50		0	1	*
Roos (4)	14	11.1	Preparation from human thyroid.	0.2 (1 dose)	Dog biscuit, milk.		−0.68		16	1	*
	15	11.2	" " "	0.2 (1 ")	" " "		−1.06		27	1	*

Voit (5)	1	19	Fresh gland.	10 (4 doses)	Meat.	−0.29		6		4	
	1	18	" "	10 (2 ")	No food.			25		2	
	1	18	Iodothyrin.†	10 (5 ")	Meat.	−1.03		4		5	*
	1	18	"	10 (2 ")	No food.			19		2	
Schöndorff (6)	1	25	Tablets.‡ Fresh gland. Dried gland.	5–10 tablets. 12 gm. fresh gland or 1 gm. dried gland daily.	Horse flesh. 30 gm. N daily.	−0.12		2		24	Same dog throughout.
	2	25		20 tablets daily.	" " " "	−1.30		6		23	
	3	25		20 " "	" " " "	−0.40		3		30	
	4	23		20–30 tablets daily.	" " " "	Positive.		0		32	
	5	23		20 tablets; 7 gm. thyroiodine; 2 days.		"		0		13	Effect gone.
Georgiewsky (7)	1	3.9	Fresh gland.	20, daily.	Horse flesh, 9 gm.	−0.78		8		5	
"	2	12.7	Cooked thyroid.	50, "	" " 11 "	−0.69		6		5	
Oswald (8)	1	11.0	Thyreoglobulin.	1 (1 dose)	Dog biscuit and milk.		−2.0		43	1	
" (9)	2	8.9	"	1 (1 ")	" " " "		−0.3		6	1	Material from goiter of pig.
	3	8.7	"	1 (1 ")	" " " "		−2.3		55	1	Material from normal pig thyroid.
	4	15.7	"	1 (1 ")	" " " "		−0.7		9	1	Material from goiter of man.

TABLE I—*Concluded.*

Experimenter.	Experiment No.	Weight of dog.	Substance.	Dose.	Food.	Average daily N balance.	N balance day of administration.	Average increase in N in urine.	Increase in N on day of administration.	Days of administration.	Remarks.
		kg.		*gm.*		*gm.*		*per cent*	*per cent*		
Underhill and Saiki (10)	1	8.0	Desiccated thyroid.	5, daily.	Meat, cracker meal, lard.	−0.7	−0.8	13	14	7	N in thyroid administered subtracted from N eliminated before calculation is made. *
	2	8.0	" "	20 (2 doses)	" " "	−0.1	+0.2	1		2	
	3	7.3	" " §	50 (1 dose)	" " "		+1.6			1	
	4	—	" "	30 (1 ")	" " "		−1.0		10	1	*
	5	—	" "	30 (1 ")	" " "		−2.1		30	1	*

* Consecutive experiments on one dog.

† 1 gm. iodothyrin preparation = 1 gm. fresh thyroid gland.

‡ Borroughs and Wellcome tablet = 0.01 N.

§ The nitrogen administered in the thyroid is over 50 per cent of the nitrogen intake, and if not all absorbed in 24 hours could account for no increase in the percentage elimination of nitrogen.

nitrogen. Powdered veal containing 10 to 15 per cent nitrogen was used for the control animals. The 24 hour collections from the metabolism cages, the cage washings, the bladder urine, and the bladder washings were combined and diluted to 2 liters. 5 cc. of diluted urine were taken for nitrogen determinations by the Kjeldahl method and run in duplicate. The records begin after several preliminary collections.

Dog 1,843.

Date.	Dose.	Total N.	Weight.
1918		*gm.*	*kg.*
Jan. 12	300 cc. sugar solution daily.	1.7	10.48
" 13		1.6	10.43
" 14		1.6	10.17
" 15		1.5	10.00
" 16	0.5 gm. thyroid.	1.4*	9.97
" 17	0.5 " "	1.6	9.80
" 18	0.5 " "	1.6	9.78
" 19	0.5 " "	1.6	9.58
" 20	0.5 " "	1.7	9.35
" 21	0.5 " "	1.9	9.12
" 22	0.5 " "	1.8	9.04
" 23		1.9	8.93
" 24		1.6	8.84
" 25		1.6	8.78

* Nitrogen eliminated in the 24 hours preceding the administration of thyroid substance.

Dog 1,847.

Date.	Dose.	Total N.	Weight.
1918		*gm.*	*kg.*
Jan. 12	300 cc. sugar solution daily.	1.8	6.97
" 13		1.4	6.91
" 14		1.3	6.83
" 15		1.2	6.74
" 16	0.5 gm. thyroid.	1.3*	6.66
" 17	0.5 " "	1.7	6.57
" 18	0.5 " "	1.7	6.54
" 19	0.5 " "	1.2	6.37
" 20	0.5 " "	1.8	6.23
" 21	0.5 " "	1.9	6.06
" 22	0.5 " "	2.0	6.01
" 23		1.9	5.95
" 24		2.2	5.89
" 25		2.0	5.78

* Nitrogen eliminated in the 24 hours preceding the administration of thyroid substance.

Dog 1,849.

Date.	Dose.	Total N.	Weight.
1918		*gm.*	*kg.*
Mar. 5	200 cc. sugar solution daily.	1.4	10.12
" 6		1.4	9.86
" 7		1.5	9.78
" 8		1.4	9.69
" 9	0.5 gm. thyroid.	1.6*	9.58
" 10	0.5 " "	1.8	9.41
" 11	0.5 " "	1.8	9.18
" 12	0.5 " "	—	9.12
" 13	0.5 " "	1.9	9.01
" 14	0.5 " "	2.2	8.84
" 15		2.4	8.73
" 16		2.4	—

* Nitrogen eliminated in the 24 hours preceding the administration of thyroid substance.

Dog 191.

Date.	Dose.	Total N.	Vol. per cent CO_2.	Weight.
1918		*gm.*		*kg.*
July 6	400 cc. sugar solution daily.	3.6		16.61
" 7		3.6		16.24
" 8		3.4		16.35
" 9		2.9	59.4	16.10
" 10		3.0	65.2	15.98
" 11	1 gm. thyroid.	3.4*	61.3	15.76
" 12	1 " "	2.4	61.3	15.53
" 13	1 " "	3.9	59.6	15.19
" 14	1 " "	4.8	61.3	14.74
" 15	1 " "	5.3	59.4	14.57
" 16	1 " "	5.2	59.4	14.23
" 17	1 " "	5.4	61.4	14.17

* Nitrogen eliminated in the 24 hours preceding the administration of thyroid substance.

Dog 192.

Date.	Dose.	Total N.	Weight.
1918		*gm.*	*kg.*
July 6	300 cc. sugar solution daily.	2.4	11.36
" 7		2.2	11.51
" 8		2.3	11.25
" 9		1.8	11.03
" 10		2.0	10.91
" 11	1 gm. thyroid.	2.2*	10.88
" 12	1 " "	3.4	10.65
" 13	1 " "	2.2	10.65
" 14	1 " "	2.6	10.40
" 15	1 " "	2.5	10.14
" 16	1 " "	2.5	10.03
" 17	1 " "	2.4	9.92
" 18	2 " "	2.2	9.72
" 19	2 " "	2.4	9.69
" 20	3 " "	2.6	9.29
" 21	3 " "	3.6	8.84
" 22	3 " "	4.4	—

* Nitrogen eliminated in the 24 hours preceding the administration of thyroid substance.

Dog 17, 160.

Date.	Dose.	Total N.	Weight.
1918		*gm.*	*kg.*
Aug. 4	400 cc. sugar solution daily.	2.7	22.05
" 5		2.6	21.88
" 6		2.6	21.63
" 7		2.6	21.54
" 8	3 gm. thyroid.	2.6*	21.43
" 9	3 " "	—	21.09
" 10	3 " "	3.6	20.86
" 11	3 " "	4.2	20.63
" 12	3 " "	3.9	20.41
" 13		4.3	20.07
" 14		4.2	19.84
" 15		3.2	19.73
" 16		3.6	19.73
" 17		2.9	19.61

* Nitrogen eliminated in the 24 hours preceding the administration of thyroid substance.

Dog 1,860.

Date.	Dose.	Total N.	Weight.
1918		*gm.*	*kg.*
Aug. 4	400 cc. sugar solution daily.	3.6	22.62
" 5		3.6	22.14
" 6		3.0	22.22
" 7		3.0	21.82
" 8	3 gm. thyroid.	2.9*	21.77
" 9	3 " "	—	21.65
" 10	3 " "	3.6	21.37
" 11	3 " "	4.0	21.03
" 12	3 " "	3.6	20.63
" 13	3 " "	4.4	20.18
" 14		5.4	19.50
" 15		6.0	20.12
" 16		4.8	19.84
" 17		3.1	19.50

* Nitrogen eliminated in the 24 hours preceding the administration of thyroid substance.

Dog 1,843.

Date.	Dose.	Total N.	Weight.
1917		*gm.*	*kg.*
Oct. 12	400 cc. sugar solution daily.	1.5	9.66
" 13		1.3	9.55
" 14		1.3	9.63
" 15	10 gm. thyroid.	1.3*	9.52
" 16	10 " "	1.7	9.38
" 17	10 " "	2.0	9.04
" 18	10 " "	2.1	8.87
" 19	10 " "	2.3	8.56
" 20		2.7	8.27
" 21		2.4	8.16
" 22		2.4	—
" 23		2.0	—

* Nitrogen eliminated in the 24 hours preceding the administration of thyroid substance.

Dog 1,849, Control.

Date.	Dose.	Total N.	Weigh.
1918		*gm.*	*kg.*
Jan. 13	300 cc. sugar solution daily.	2.4	8.73
" 14		2.1	8.52
" 15		1.9	8.52
" 16	0.4 gm. meat powder.	1.9	8.38
" 17	0.4 " " "	1.9	8.30
" 18	0.4 " " "	1.9	8.17
" 19	0.4 " " "	1.8	8.15
" 20		1.9	7.95
" 21		1.9	7.86
" 22		2.2	7.75

Dog 1,867, Control.

Date.	Dose.	Total N.	Vol. per cent CO_2.	Weight.
1918		*gm.*		*kg.*
Mar. 5	300 cc. sugar solution daily.	2.2	53.8	9.29
" 6		2.3	57.4	9.01
" 7		2.5	51.6	8.95
" 8		—		8.84
" 9	0.5 gm. meat powder.	2.1		8.87
" 10	0.5 " " "	1.7		8.78
" 11	0.5 " " "	1.6		8.73
" 12	0.5 " " "	1.4		8.73
" 13	0.5 " " "	1.4		8.61
" 14	0.5 " " "	1.2		8.53
" 15	0.5 " " "	1.3	53.9	8.44
" 16	0.5 " " "	1.3	53.8	—

Dog 1,879, Control.

Date.	Dose.	Total N.	Weight.
1918		*gm.*	*kg.*
Jan. 13	300 cc. sugar solution daily.	1.4	6.37
" 14		1.5	6.20
" 15		1.4	6.09
" 16	0.5 gm. meat powder.	1.3	6.01
" 17	0.5 " " "	1.3	5.98
" 18	0.5 " " "	1.2	5.98
" 19	0.5 " " "	1.3	5.92
" 20	0.5 " " "	1.3	5.84
" 21	0.5 " " "	1.3	5.75
" 22	0.5 " " "	1.1	5.69
" 23	0.5 " " "	1.1	5.61
" 24	0.5 " " "	1 2	5.52

* Nitrogen eliminated in the 24 hours preceding the administration of thyroid substance.

Dog 193, Control.

Date.	Dose.	Total N.	Vol. per cent CO_2.	Weight.
1918		*gm.*		*kg.*
July 5	300 cc. sugar solution daily.	2.5		10.43
" 6		2.4		10.17
" 7		2.3		10.09
" 8		2.3		10.00
" 9		2.1	61.3	9.95
" 10		1.4	55.6	9.69
" 11		2.6	59.4	9.63
" 12	1 gm. meat powder.	2.2	59.4	9.41
" 13	1 " " "	2.2	53.8	9.29
" 14	1 " " "	2.2	59.5	9.24
" 15	1 " " "	2.1	59.5	9.12
" 16	1 " " "	1.2	61.4	9.07
" 17	1 " " "	3.1	61.4	—

Dog 1,859.			Dog 1,821 (control).		
Date.	Dose.	Vol. per cent CO_2.	Date.	Dose.	Vol. per cent CO_2.
1918			*1918*		
Aug. 14	336 cc. sugar solution daily.	59.5	Aug. 14	400 cc. sugar solution daily.	57.6
" 15		62.4	" 15		—
" 16		63.3	" 16		58.5
" 17		63.3	" 17		59.5
" 19		63.3	" 19		65.3
" 20		57.6	" 20		58.6
" 21	3 gm. thyroid.	57.7	" 21		59.6
" 22	3 " "	58.6	" 22		59.5
" 23	3 " "	56.6			

DISCUSSION.

These experiments would indicate that studies of the basal nitrogen metabolism of dogs receiving sugar solutions over a period of about 2 weeks is sufficient to indicate whether or not nitrogen metabolism is accelerated by thyroid preparations administered during this period.

The summary of results showing the average nitrogen figures for 3 days preceding thyroid feeding and for the days of administration including two subsequent days,[1] would indicate that a daily dose of 0.05 to 0.1 gm. of desiccated thyroid mixture per kilo of body weight is a dose producing a marked effect upon nitrogen elimination and a somewhat greater percentage loss in weight than is found in the control animals.

The condition of the animals remained excellent throughout the experiments. The carbon dioxide-combining power of the plasma of several of the animals studied was not affected by the thyroid substance given. Fresh thyroid glands were not fed as a control upon these experiments, so at present no discussion of

Summary of Results.

Experiment No.	Dog No.	Dose.	Weight.	Average daily N for 3 days previous to administration.	Average daily N for days of administration + 2 days following.	Increase in N.	Loss in wt. in 3 days before administration.	Loss in wt. in first 3 days of administration.	Loss in wt. in second 3 days of administration.
Thyroid preparation administered.									
		gm. per kg.	*kg.*	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*
1	1,843	0.05	10	1.5	1.7	13	4.4	3.9	5.6
2	1,849	0.05	10	1.5	2.0	33	2.8	4.8	4.2
3	191	0.07	15	3.1	4.5	45	3.6	6.4	3.8
4	1,847	0.10	6	1.2	1.8	50	3.6	4.3	5.6
5	192	0.1–3	10	2.0	2.8	40	3.2	4.4	4.6
6	17,160	0.15	22	2.6	3.9	50	2.0	3.7	3.3
7	1,860	0.15	22	3.0	4.5	50	1.2	3.4	7.2
8	1,843	1.00	9	1.3	2.2	69	1.4	6.8	8.0
Meat powder administered.									
1	1,867	0.05	9	2.3	1.4	Decrease.	1.5	1.6	3.3
2	1,849	0.04	8	1.9	1.9	0	4.0	2.7	4.9
3	1,879	0.1	6	1.4	1.2	Decrease.	5.6	1.5	3.8
4	193	0.1	10	2.0	2.1	4	5.4	3.1	—

[1] The calculations for average percentage increase in nitrogen elimination in the tabular summary of the literature are made on this basis.

the question as to whether the effect of desiccated thyroid upon metabolism is physiological or toxicological can be made.

CONCLUSION.

Nitrogen elimination in the dog receiving only sugar solutions may be increased approximately 50 per cent by the administration, during a 5 to 7 day period, of commercial desiccated thyroid gland in doses of 0.10 to 0.15 gm. per kilo of body weight.

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THE ACID-BASE BALANCE IN ANIMAL NUTRITION.

I. THE EFFECT OF CERTAIN ORGANIC AND MINERAL ACIDS ON THE GROWTH, WELL BEING, AND REPRODUCTION OF SWINE.*

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INTRODUCTION.

The question of the balance between acid- and base-forming mineral elements in foodstuffs has figured more or less prominently in discussions of rations for both man and animals during the last decade. Based principally on the work of Forbes,[1] Sherman and Gettler,[2] and Kastle,[3] there is a tendency to emphasize the necessity for a balance between potential acid and base in the mineral constituents of rations. In this preliminary discussion human dietary standards will figure, as well as rations for animals, but in reporting our conclusions we shall avoid the error of applying data obtained with one species to other species.

Much of the early literature on this subject is cited by Forbes and by Sherman and Gettler. The former's discussion of the acid-base balance is largely theoretical, and much of it is based upon notions which have since been found incorrect. The analyses of Sherman and Gettler, which are the first to show accurately the true relations of the principal mineral elements in foodstuffs, are valuable and their interpretation of their metabolism experiments is conservative, but others have often overemphasized the necessity of a balance such as they suggest. We wish to point out fallacies in the reasoning of those who insist on the necessity of this balance of ash elements.

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* Published with the permission of the Director of the Iowa Agricultural Experiment Station. Preliminary report read at the Kansas City meeting of the American Chemical Society, April 12, 1917.

[1] Forbes, E. B., *Ohio Agric. Exp. Sta., Bull. 207*, 1909.

[2] Sherman, H. C., and Gettler, A. O., *J. Biol. Chem.*, 1912, xi, 323.

[3] Kastle, J. H., *Am. J. Physiol.*, 1908, xxii, 284.

The interpretation of experiments comparing natural foodstuffs with varying acid and base content is difficult, because the character and composition of the ash, aside from its acid or basic properties, may have much influence on the results, as has been shown by McCollum,[4] and by Osborne and Mendel.[5] Other elements in the ration, such as the character of the proteins, may be limiting factors. A prime essential in fundamental nutrition experiments is that the unknown factors as far as possible should be limited to one. Then only is complete interpretation and generalization possible. The tendency has often been to accept too readily as a reason for unfavorable results the potential acid character of the ration, when other possibilities are present. For example, in natural feedingstuffs such as oats, wheat, and corn, the mineral or protein content is often deficient in quality, yet conclusions are sometimes drawn solely on the basis of the excess of acid-forming elements. Weiser[6] fed pigs on corn alone, which is very low in calcium, obtaining a positive nitrogen and a negative calcium balance. Calcium was added in the form of carbonate and the calcium balance became positive. Funk[7] implies that this effect obtained by Weiser is due to the correction of the acidity of the ration. In his own work with rabbits Funk finds that a diet of oats alone causes death in 30 to 70 days, and thinks the failure of the ration due to the excess of acid-forming mineral elements in the oats. He, as well as others who have fed rabbits on an exclusive oat diet, appears to disregard the fact that herbivorous animals, including rabbits, require roughage as a constituent of the ration. Unfavorable results from an abnormal as well as incomplete diet are then interpreted as being due to the potential acidity of the oats. It is noteworthy that Funk finds that rats, which are omnivorous, can be maintained on oats and white bread for a long time, although this diet fails to produce growth in young rats. That the failure in growth is not due to the acidity of the diet is shown by the fact that the addition of sodium bicarbonate had no beneficial action. Other cases might be cited from the literature, but the foregoing illustrations show the tendency to consider acidosis as a frequent cause of nutritional troubles.

Acidosis is variously defined. As most generally accepted it is a pathological condition which occurs in diabetes, nephritis, surgical cases, and as a result of poisoning or starvation. In infants it is sometimes a result of too high fat feeding, but it is then clearly due to a limited capacity to digest and assimilate fats. We dislike to use this term, which really belongs to pathology, in discussing normal nutrition. Indeed the widespread tendency

[4] McCollum, E. V., and Davis, M., *J. Biol. Chem.*, 1915, xxi, 615.

[5] Osborne, T. B., and Mendel, L. B., *Carnegie Institution of Washington, Publication 156*, pt. ii, 1911; *J. Biol. Chem.*, 1918, xxxiv, 131.

[6] Weiser, S., *Biochem. Z.*, 1912, xliv, 279.

[7] Funk, C., *J. Biol. Chem.*, 1916, xxv, 409.

to write into the literature of normal nutrition data obtained from drug administration, pathological cases, and other abnormal conditions is to be deprecated. Drug administration is of value, and experiments on diabetic patients are of importance in the study of diabetic acidosis, but unfortunately the data obtained in such studies are widely accepted as showing the limited tolerance of the animal organism for acid, disregarding the fact that the organisms experimented on are in an abnormal or pathological condition.

The protective mechanism of the body against acids has been thoroughly studied by Henderson.[8] That the organism has considerable tolerance for acid has been shown by McCollum.[9] He reported satisfactory growth from an early age and normal reproduction in rats fed exclusively on egg yolk, which Sherman and Gettler find to have a potential excess acidity of 26.7 cc. normal solution per 100 gm. McCollum and Hoagland[10] have shown that a pig on a nitrogen-free diet plus mineral acids can neutralize a large part of the acid with ammonia and maintain tissue neutrality. At the same time they found that the creatinine nitrogen in the urine remained constant, suggesting that the extra nitrogen catabolized to neutralize the ingested acid probably originated elsewhere than in muscle tissue. Steenbock, Nelson, and Hart,[11] using swine and calves on a normal level of protein intake, found that the rise in urinary ammonia nitrogen was not accompanied by a corresponding rise in the total urinary nitrogen, but that there was a commensurate drop in the urea nitrogen. They concluded that on a normal protein intake the excess of acid-forming mineral elements which might exist in natural foods should exercise no deleterious effect on protein storage.

The possibility is thus established that an animal may be able to grow and complete its normal cycle of life on a ration which contains an excess of acid-forming mineral elements, but which is

[8] Henderson, L. J., *J. Biol. Chem.*, 1911, ix, 403; *Science*, 1913, xxxvii, 389; 1917, xlvi, 73, and other papers.

[9] McCollum, E. V., *Am. J. Physiol.*, 1909–10, xxv, 127.

[10] McCollum, E. V., and Hoagland, D. R., *J. Biol. Chem.*, 1913–14, xvi, 299.

[11] Steenbock, H., Nelson, V. E., and Hart, E. B., *J. Biol. Chem.*, 1914, xix, 399.

otherwise satisfactory. It has been shown that animals of all types of feeding habits may divert part of the nitrogen which would otherwise appear in the urine as urea for the production of ammonia, thereby neutralizing a large part of the acids ingested or formed in metabolism. Sherman and Gettler,[2] however, with a human subject on acid and alkaline diets, found that the extra urinary acidity and ammonia failed to account for all the extra acidity of the acid diet. This fact may be the reason for the tendency to consider a balance between acids and bases necessary. It is possible, however, that the greater part of this remainder may be accounted for in the feces without any serious loss of bases from the body. Inasmuch as bacterial residues constitute a large part of the mass of feces, it is conceivable that some sulfur and phosphorus is excreted combined with protein and in other unoxidized forms. Also, in growth, a considerable amount is stored in the form of proteins, phosphatides, and nuclear material Thus it seems possible that the excess acid of natural foods may be accounted for without serious loss of base from the body, by means of ammonia production, acid phosphate excretion, and the other factors just mentioned. Of course we refer to *normal* nutrition. The only way to determine definitely whether or not a balance between acid- and base-forming mineral elements is necessary is to test the ability of animals to maintain growth and well being and to reproduce normally on a satisfactory ration with a considerable excess of acid. Inasmuch as swine are convenient experimental animals and grow so rapidly that their weight is quadrupled in a few months, they have been used in our experiments. Swine are generally fed largely on cereals, which always carry an acid ash, and it is important to know for practical reasons whether their rations should be balanced with bases.

EXPERIMENTAL.

These experiments were initiated with the purpose of testing the ability of swine to metabolize successfully the lactic and acetic acids of silage. In the course of our silage investigations at the Iowa station, it has been found possible to make a satisfactorily preserved silage from rape (*Brassica napus*).[12] As or-

[12] Lamb, A. R., and Evvard, J. M., *J. Agric. Research*, 1916, vi, 527.

dinary corn silage is too fibrous to be utilized efficiently by swine, silage has not been well tested in swine feeding. In this experiment lactic, acetic, and sulfuric acids were fed in equivalent amounts to separate lots of pigs, the mineral acid being fed for comparison with the organic acids. Since the lot receiving sulfuric acid grew practically as well as the control lot, the question of the balance of mineral acids and bases was raised, and the work continued on that subject.

The plan of the first experiment was as follows. Eight pigs, all from the same litter, about 100 days old and weighing 50 to 60 pounds, were divided into four lots of two pigs each. All were healthy and thrifty, and similar in appearance and condition. All were fed twice daily the same basal ration, well tried and satisfactory, consisting of 80 per cent ground corn, 15 per cent meat meal tankage (which contained 60 per cent protein), and 5 per cent standard wheat middlings. The amount fed to each lot was regulated by the amount the lot eating least would eat each day. Thus each lot received the same amount of feed—an important matter since the gains in weight of the lots were to be compared. Water in the drinking troughs was allowed *ad libitum*. With this exception the lots were treated similarly in every respect until near the close of the experiment. The mixed feed was mixed with an equal weight of water in the feeding troughs, and the acids, in the form of normal solutions, were thoroughly mixed with the moist feed.

Lot I was used as a control throughout the experiment, Lot II was fed sulfuric acid, Lot III an equivalent amount of lactic acid, and Lot IV an equivalent amount of acetic acid. The total length of the experiment, except for Lot II which was continued longer on sulfuric acid, was seven 30 day periods. The periods and the amounts of acid added to the ration are shown in Table I.

The rate of growth of the four lots was quite uniform in spite of the acid additions. If the growth curves were plotted on a scale small enough to be suitable for reproduction here, it would be difficult to distinguish any difference. Neither growth nor well being appears to have been interfered with appreciably by the acids added to the ration. Table II gives the weights of the animals by 30 day periods. Weights were taken every 10 days. While Lot II weighed considerably less than the control lot at

TABLE I.

Plan of Growth Experiment, Showing Amounts of Normal Acid Fed.

Period No.	Periods.	Lot I	Lot II	Lot III	Lot IV	Remarks.
I	*days* 30 (control)	Basal ration only.	Basal ration only.	Basal ration only.	Basal ration only.	Began Oct. 4, 1916. All lots fed same amount of feed.
II	30	Basal ration only.	100 cc. N sulfuric.	100 cc. N lactic.	100 cc. N acetic.	
III	10	Basal ration only.	200 cc. N sulfuric.	200 cc. lactic.	200 cc. acetic.	
	10	" " "	300 " " "	300 " "	300 " "	
	10	" " "	400 " " "	400 " "	400 " "	
IV	30	Basal ration only.	500 cc. sulfuric.	500 cc. lactic.	500 cc. acetic.	
V	30	Basal ration only.	500 cc. acetic.	500 cc. sulfuric.	500 cc. lactic.	Note change in acids.
VI	30	Basal ration only.	500 cc. sulfuric.	500 cc. lactic.	500 cc. acetic.	Lots fed according to appetite last 50 days of experiment.
VII	30 (control)	Basal ration only.	No acid.	No acid.	No acid.	

the close of the experiment, this was partly due to the fact that during the last 50 days all lots were fed according to appetite. It should be noted that at the beginning of Period VI, when all lots were being fed the same, the differences between the lots in average weights were less than the variations between individuals within the lots.

No difficulty was experienced in feeding the acid in the manner described above. At one time only during the experiment, the animals in Lot II lost appetite and did not eat well for a few days. This was soon after being changed from the acetic acid ration back to sulfuric acid. It merely calls attention to the difficulty under

TABLE II.

Weights of Animals at Beginning of Each 30 Day Period and at End of Experiment.

Period.	Lot I		Lot II		Lot III		Lot IV	
	Animal 460	Animal 465	Animal 461	Animal 463	Animal 462	Animal 464	Animal 466	Animal 467
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
I	66	51	63	55	53	61	59	57
II	95	80	96	80	89	89	77	98
III	127	117	126	114	116	115	105	136
IV	158	157	164	147	157	142	139	176
V	194	197	196	183	199	171	177	216
VI	217	238	238	210	223	200	219	241
VII	260	272	255	244	269	249	247	287
Final weight....	327	327	304	295	303	306	306	336

which these animals labored when they were obliged to take into the stomach 250 cc. of *normal* acid at each feeding time. There was apparently, however, little interference with digestion, as shown by the growth curves. It appears probable that it is at least as difficult for the organism to neutralize acid thus ingested as to neutralize acid produced in the tissues by gradual oxidation. When acid is given by mouth the greater part of it must be neutralized at once by whatever material is most convenient, and readjustments made later. It is possible that this is more wasteful of fixed bases than the neutralization of acid produced gradually in the tissues.

In addition to the superimposed acid, the basal ration itself contained an excess of acid-forming mineral elements amounting to about 2.55 cc. of normal solution per 100 gm. of ration, or about 60 cc. normal solution per pig per day. It should also be noted that the basal ration we fed was nearly as high in potential acidity as would be possible in a satisfactory ration for growing pigs. The amount of acid superimposed upon this ration was more than eight times the amount of the natural potential acidity of the feed. Under these circumstances slight variations in the rate of growth, etc., are of little importance when the interpretation is made from the standpoint of natural rations and their low potential acidity.

TABLE III.

Comparative Data.

	Lot I	Lot II	Lot III	Lot IV
	lbs.	*lbs.*	*lbs.*	*lbs.*
Average initial weight per pig	58.6	59.0	57.0	58.3
" final weight	327.4	299.4	304.4	320.7
" daily gain per pig	1.28	1.14	1.18	1.25
" " feed	5.62	5.26	5.51	5.57
" " " per 100 lbs. live wt.	3.15	3.05	3.24	3.12
" feed to produce 100 lbs. gain	439.4	460.0	467.4	445.8
" daily gain per pig for first five periods only	1.13	1.10	1.03	1.14

Some significant comparative data showing the average daily gain, etc., in each lot, are given in Table III. The table shows clearly the relative gains in weight of the four lots. Lot I (control) made a little better gain and somewhat more economically than the others. The record made by Lot I was exceptionally good. Lot II (sulfuric acid) made the least daily gain, but the difference is not very great, perhaps not much greater than variations due to individuality. It should also be noted that Lot II consumed less feed per day and per 100 pounds live weight. All lots received the same amount of feed until the last 50 days, when each lot was allowed as much feed as it would readily consume. Part of the variations shown are due to this change in treatment, since the lot fed sulfuric acid would

not consume so much feed as the others, presumably on account of the admixture of strong acid.

The average daily gain per pig for the first five periods only, when all lots were being fed the same amount of feed, shows more accurately the performance of the various lots than the average for the whole experiment and checks well with other averages for the various periods. It will be noted that the rates of gain in Lots II and III are still a little below Lot IV as well as below the control lot. However, there are other factors to be taken into consideration in the case of Lot II which have already been discussed.

The organic acids fed Lots III and IV were apparently oxidized, as early morning samples of urine taken at intervals showed no higher ammonia content than the urine of the control lot.

TABLE IV.

Animal No.	Lot	pH
460	I	7.2
465	I	7.1
461	II	7.1
463	II	7.0
464	III	7.1
467	IV	7.2

Growth and well being in these lots were entirely comparable with the control lot. No change in the growth curves was noted when the acids were interchanged in Period V (Table I). Further data on these questions are given in the second paper of this series.

Near the end of the last acid-feeding period, samples of blood were taken from several of the animals and the hydrogen ion concentration was determined by the method of Levy, Rowntree, and Marriott.[13] The results (Table IV) show definitely that neither the organic nor mineral acids used disturbed the reaction of the blood, and demonstrate the efficiency of the protective mechanism against acids.

[13] Cf. Hawk, P. B., Practical physiological chemistry, Philadelphia, 5th edition, 1916. 288.

Reproduction Test.

The two animals in Lot II, after the last control period of the preceding experiment (see Table I), and after a 10 day control period during which they were treated for worms (using santonin and calomel) with negative results, were continued on the same ration plus 500 cc. of normal sulfuric acid, just as before. The acid feeding began again on May 12, 1917, and the two animals Nos. 461 and 463, were bred on April 19 and June 5, respectively. Except for a few feeds during the extremely hot weather these animals ate well, remained in thrifty condition, and each received 500 cc. of normal acid daily.

On August 12, Animal 461 farrowed eight strong vigorous pigs which suckled normally for more than a week, when seven of them died within a day or two. The last one was killed accidentally when nearly a month old. The possibility of some toxic quality in the milk is not excluded, but seems unlikely. We are obliged to consider these results inconclusive.

Animal 463 farrowed three vigorous pigs on September 27. One was transferred to another litter to check if possible the influence of the milk. Unfortunately the other sow killed this pig by lying on it. Of the other two, one died when nearly 4 weeks old. Postmortem examination[14] showed that death was due to pericarditis resulting from infection, which could not be directly ascribed to the ration. The last pig passed successfully through the suckling period, and weighed 33 pounds when 60 days old. The results in this case were more favorable, although not entirely successful. It is apparent, however, that a strongly acid ration did not interfere with prenatal development at least, and the trouble may have been due to some cause outside the ration.

Qualitative tests made on the milk of Sow 463 showed it to be neutral in reaction, normal in appearance and odor, with a trace of sulfates, and a positive test for calcium in the milk serum. It should also be noted that the urine of this sow showed no evidence of glycosuria or albuminuria.

[14] Postmortem examination was made by Dr. W. W. Dimock, Professor of Veterinary Pathology at Iowa State College.

Postmortem examination was not made of the pigs of the first litter, nor was it possible to make slaughter tests of the two sows, nor to examine the character of their bones.[15] The bones of the young pigs, however, were all above the average in size, as there was an abundance of calcium in the meat meal tankage of the ration. This reproduction test will be repeated at our earliest opportunity.

While the results in growth on the sulfuric acid ration were successful and the results in reproduction fairly good, it must be noted that the ration was a good one, containing an abundance of protein and mineral matter, even though the excess of mineral elements was on the acid side. This ration was not better than should be fed to insure good results, but was merely a satisfactory ration. If the ration were lacking in some essential, or poor in quality, the results might be different. These results show, however, that *if the other elements in a natural ration are satisfactory, it is not necessary to balance the acid and basic mineral elements for growing swine.* No suitable combination of natural feeds would contain nearly as much potential mineral acid as was fed in this experiment, during which the animals in Lot II increased in weight from 88 to an average of 370 pounds and successfully produced young.

The metabolism of these acids and the question of the possible loss of fixed bases from the body, are discussed in the second paper of this series.

SUMMARY.

The necessity for balancing the potential acid and base of rations has been in dispute, but nutritional failures are often ascribed to excess acidity when other causes are not wholly excluded.

Four lots of two pigs each were fed equal amounts of a good basal ration. Three of the lots received sulfuric, lactic, and acetic acids respectively, in amounts up to 500 cc. of normal solution per pig per day. This continued for 150 days of acid feeding. The three acid-fed lots grew practically as rapidly as the fourth lot, which was a control, and remained in equally good condition.

[15] Postmortem on a pig fed sulfuric acid in a later experiment showed sternum, ribs, and skull normal. Bones were flinty and hard with less marrow than control.

The organic acids were apparently completely oxidized, and the sulfuric acid was neutralized without apparent harm or significant effect on growth.

The two pigs fed sulfuric acid were continued on the same ration for 4 to 6 months longer and successfully produced young. Either the excessive amount of acid fed or some other factor, however, prevented the successful rearing of the young.

THE ACID-BASE BALANCE IN ANIMAL NUTRITION.

II. METABOLISM STUDIES ON THE EFFECT OF CERTAIN ORGANIC AND MINERAL ACIDS ON SWINE.*

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In Paper I of this series it was reported that swine apparently possess the ability to maintain normal growth and well being when ingesting considerable amounts of lactic, acetic, or sulfuric acids with the ration. This fact has made it necessary to secure more definite information concerning the fate of these acids in the animal body.

While it is known that lactic and acetic acids are oxidizable, several observers have reported traces in normal urine, thus suggesting a limited ability to oxidize them. There is some evidence that lactic acid is an intermediate product in the oxidation of glucose. Embden[1] has shown it to be closely related with alanine and pyruvic acid in the organism. While lactic acid appears in the urine in cases of oxygen starvation, especially in phosphorus poisoning or asphyxiation, it appears under normal conditions to be completely oxidized. Acetic acid is probably completely oxidized, although the mechanism of its oxidation is not known. It appears that these acids should not tax the neutralizing powers of the organism. However, large quantities of these two acids have not hitherto been fed to an animal in a metabolism cage.

In the case of unoxidizable mineral acids, while the mechanism of neutralization has been found to be efficient, various harmful possibilities, such as the withdrawal of fixed bases from the body,

* Published with the permission of the Director of the Iowa Agricultural Experiment Station.

[1] For bibliography on this subject see Dakin, H. D., Oxidations and reductions in the animal body, London, 1912.

have been suggested.[2] The influence of the considerable amounts of ammonium salts formed when acids are neutralized has sometimes been considered detrimental. Voegtlin and King reported[3] that intravenous injection of ammonium salts (presumably in dogs) produced symptoms of acid intoxication, which were relieved by the injection of calcium salts. Underhill[4] also found some evidence of toxicity when considerable amounts of ammonium chloride were given *per os*. The loss of calcium from the bones is the most serious possibility suggested with regard to the fixed bases, which may be used in neutralization. However, misleading conclusions have been drawn on this subject when the experimental animal was receiving a ration too low in calcium for proper nutrition. A single example of this will be cited. Stehle[5] reports a loss of calcium when hydrochloric acid was administered to a dog. The diet was, however, entirely inadequate in lime, as in several other experiments in the literature where animals were fed on meat alone, without bone. Givens and Mendel[6] have recently shown that neither acid nor alkali exerts any marked effect on calcium storage. In a later paper Givens[7] confirms these conclusions.

EXPERIMENTAL.

A vigorous thrifty barrow pig which weighed 60 pounds at 90 days of age at the beginning of this work was placed in a metabolism cage with a separate feeding stall, a modification of the cage used by McCollum. The ration previous to this experiment had been satisfactory. The ration we used during the first cage experiment was the same that was used in the growth experiment recorded in the preceding paper; *viz.*, ground corn 80 per cent, meat meal tankage 15 per cent, wheat middlings 5 per cent. The feed was mixed with water, and the acids were added in the same manner as in the experiment just mentioned. The pig was

[2] See discussion in Paper I of this series.
[3] Voegtlin, C., and King, I., *J. Biol. Chem.*, 1909, vi, p. xxviii.
[4] Underhill, F. P., *J. Biol. Chem.*, 1913, xv, 327 ff.
[5] Stehle, R. L., *J. Biol. Chem.*, 1917, xxxi, 461.
[6] Givens, M. H., and Mendel, L. B., *J. Biol. Chem.*, 1917, xxxi, 421.
[7] Givens, M. H., *J. Biol. Chem.*, 1918, xxxv, 241.

fed twice daily. The urine was collected every 24 hours, and the feces by 5 day periods.

The pig was placed in the cage for a preliminary period of 5 days to accustom him to the cage and the ration. The regular experimental periods which followed and the rations with additions are shown in Table I.

TABLE I.

Period No.	Period.	Average daily ration.	Acid fed daily.	Weight of animal.
		lbs.		*lbs.*
I	Control, 10 days.	1.8	None.	62.0
II	Acid, 10 days.	2.0	250 cc. N lactic.	69.5
III	" 10 "	2.0	300 " " sulfuric.	75.0
IV	" 10 "	2.0	300 " " acetic.	81.0
V	Control, 10 days.	2.0	None.	87.0

Some analytical data on the mixed ration as used are shown in Table II.

TABLE II.

H_2O	CaO	MgO	P_2O_5	N
per cent	*per cent*	*per cent*	*per cent*	*per cent*
11.95	1.12	0.25	1.74	2.85

The urinary acidity was determined by titration with phenolphthalein according to Folin. Urea and ammonia nitrogen were determined by the method of Van Slyke and Cullen. Folin's colorimetric method for creatinine and the Folin-Benedict method for creatine were used. Calcium in urine and feces was determined by McCrudden's method. Total urinary sulfur was determined by Benedict's method, and sulfate sulfur according to Folin.

The analytical data on the urine in the first experiment are given in Table III. Averages of the urinary constituents are given by periods.[8]

Since there is always more or less lag in the response of the urinary constituents to changes in the ration, a more nearly cor-

[8] Credit is due Mr. W. J. Suer for much of the analytical work reported in Table III.

TABLE III.

Urine Analysis. Experiment 1.

Period.	Date of sample.	Volume.	Specific gravity.	Reaction to litmus.	Acidity, 0.1 N.	Total N.	Urea N.	NH_3-N	Creatinine N.	Creatine N.	CaO	Total S.	Sulfate S.	Unoxidized S.*	Remarks.
	1917	*cc.*			*cc.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
I	June 29	537	1.029	Acid.	—	10.139	7.527	0.977	0.299	—	0.129	0.599	0.549	0.050	Control period.
	" 30	922	1.018	"	244	12.023	9.207	1.203	0.338	0.082	0.208	0.770	0.680	0.090	
	July 1	1,770	1.010	Neutral.	186	12.532	9.080	1.675	0.355	0.059	0.183	0.812	0.747	0.065	
	" 2	1,155	1.012	Acid.	295	11.573	8.384	1.588	0.343	0.000	0.318	0.714	0.666	0.048	
	" 3	830	1.016	"	282	9.213	6.015	1.459	0.293	0.040	0.267	0.619	0.575	0.044	
	" 4	640	1.020	"	237	8.448	5.720	1.663	0.267	0.096	0.227	0.557	0.509	0.048	
	" 5	525	1.024	"	304	7.718	4.966	1.414	0.286	0.107	0.171	0.516	0.469	0.047	
	" 6	560	1.024	"	319	10.248	7.259	1.443	0.301	0.108	0.199	0.632	0.565	0.067	
	" 7	640	1.021	"	294	10.957	7.974	1.423	0.321	0.064	0.173	0.612	0.533	0.079	
	" 8	690	1.025	Neutral.	262	10.805	7.798	1.329	0.325	0.156	0.196	0.680	0.595	0.085	
Average....		827	1.020		270	10.37	7.39	1.42	0.313	0.079	0.207	0.651	0.589	0.062	
II	July 9	780	1.022	Acid.	288	11.684	8.509	1.332	0.336	0.133	0.221	0.632	0.573	0.059	Began 200 cc. N lactic daily.
	" 10	795	1.021	"	294	10.653	7.509	1.560	0.332	0.137	0.258	0.647	0.559	0.088	
	" 11	605	1.025	"	314	9.995	6.994	1.320	0.308	0.226	0.231	0.649	0.582	0.057	
	" 12	540	1.028	"	227	10.130	7.082	1.409	0.270	0.121	0.238	0.534	0.513	0.021	
	" 13	595	1.025	"	321	10.032	7.338	1.215	0.338	0.086	0.274	0.620	0.563	0.057	
	" 14	575	1.029	"	282	10.212	7.686	1.079	0.346	0.096	0.218	0.660	0.580	0.080	Began 300 cc. N lactic.
	" 15	540	1.028	"	227	10.217	7.597	1.146	0.319	0.042	0.219	0.591	0.548	0.043	
	" 16	543	1.029	"	282	10.208	7.504	1.236	0.308	0.024	0.213	0.503	0.468	0.035	
	" 17	562	1.029	"	337	10.487	7.532	1.342	0.313	0.098	0.243	0.628	0.554	0.074	
	" 18	640	1.028	"	346	10.918	7.946	1.122	0.356	0.123	0.218	0.684	0.590	0.094	
Average....		617	1.026		292	10.45	7.54	1.28	0.323	0.109	0.233	0.615	0.553	0.061	

III	July 19	970	1.022	Acid.	310	9.603	5.767	2.140	0.313	0.097	0.393	2.564	2.388	0.176	Began 300 cc. N H_2SO_4 daily.
	" 20	725	1.032	"	370	9.541	6.110	2.424	0.312	0.040	0.612	3.961	3.666	0.295	
	" 21	1,145	1.022	"	332	10.534	5.463	3.142	0.382	0.026	0.670	4.128	3.813	0.315	
	" 22	810	1.030	"	437	11.259	6.138	3.252	0.406	0.021	0.853	4.269	4.002	0.267	
	" 23	950	1.028	"	437	11.647	6.118	3.469	0.402	0.000	0.957	4.694	4.464	0.230	
	" 24	885	1.027	"	416	11.293	5.680	3.558	0.410	0.014	0.950	4.446	4.387	0.059	
	" 25	1,345	1.021	"	390	12.912	6.154	4.196	0.414	0.110	0.986	5.067	4.627	0.440	
	" 26	995	1.026	"	507	12.139	6.394	4.296	0.415	0.120	0.773	5.386	4.544	0.842	
	" 27	740	1.027	"	340	9.235	4.914	3.498	0.349	0.071	0.259	3.632	3.404	0.228	
	" 28	1,340	1.024	"	456	15.812	7.076	6.566	0.552	0.169	0.433	6.098	5.494	0.604	
Average		991	1.026		399	11.40	5.98	3.65	0.396	0.067	0.689	4.425	4.079	0.346	
IV	July 29	1,200	1.020	Acid.	408	12.648	6.498	4.657	0.467	0.107	0.296	3.133	2.765	0.368	Began 300 cc. N acetic daily.
	" 30	1,510	1.011	"	242	10.691	5.886	3.822	0.314	0.140	0.109	0.867	0.687	0.180	
	" 31	1,635	1.012	"		10.693	6.181	2.197	0.370	0.212	—	0.800	0.623	0.177	
	Aug. 1†	—	—	—	—	—	—	—	—	—	—	—	—	—	
	" 2	858	1.022	Acid.		12.441	8.048	2.360	0.344	0.254	0.391	0.922	0.685	0.237	
	" 3	1,005	1.016	"		12.080	7.904	2.136	0.324	0.302	0.290	0.635	0.505	0.130	
	" 4	1,080	1.022	"		11.426	7.212	1.678	0.397	0.412	0.219	0.660	0.574	0.086	
	" 5	845	1.021	"		11.204	7.370	1.488	0.288	0.210	0.171	0.607	0.474	0.133	
	" 6	650	1.024	"		10.894	7.404	1.478	0.311	0.051	0.153	0.737	0.625	0.112	
	" 7	885	1.020	"		12.408	8.242	1.601	0.355	0.236	0.181	0.721	0.578	0.143	
Average		1,074	1.019			11.61	7.19	2.38	0.352	0.214	0.226	1.009	0.835	0.174	

TABLE III—*Concluded.*

Period.	Date of sample.	Volume.	Specific gravity.	Reaction to litmus.	Acidity, 0.1 N.	Total N.	Urea N.	NH_3-N	Creatinine N.	Creatine N.	CaO	Total S.	Sulfate S.	Unoxidized S.*	Remarks.
	1917	*cc.*			*cc.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
V	Aug. 8	720	1.025	Acid.		13.032	9.001	1.482	0.433	0.120	0.226	0.617	0.524	0.093	Control period.
	" 9	812	1.023	"		10.848	6.921	1.582	0.383	0.171	0.280	0.721	0.606	0.115	
	" 10	857	1.020	"		10.181	6.591	1.385	0.362	0.144	0.130	0.649	0.547	0.102	
	" 11	905	1.021	"		10.245	6.614	1.444	0.339	0.175	0.145	0.701	0.603	0.098	
	" 12	713	1.023	"		9.982	6.009	1.697	0.278	0.135	0.123	0.666	0.580	0.086	
	" 13	633	1.027	"		10.836	7.129	1.379	0.294	0.147	0.102	0.657	0.557	0.100	
	" 14	957	1.019	"		11.867	7.608	2.039	0.337	0.128	0.080	0.760	0.652	0.108	
	" 15	710	1.023	"		10.465	6.799	1.630	0.296	0.223	0.068	0.670	0.571	0.099	
	" 16	540	1.024	"		10.214	7.141	1.178	0.271	0.155	0.101	0.619	0.552	0.067	
	" 17	620	1.024	"		11.172	7.635	1.601	0.306	0.080	0.111	0.711	0.612	0.099	
Average		747	1.023			10.88	7.15	1.54	0.330	0.148	0.137	0.677	0.581	0.097	

All data on 24 hour output.

* By difference.

† Part of sample lost.

rect average for each period has been obtained by taking the last 8 days of each 10 day period. These figures are shown in Table IV.

It will be noted that neither the lactic nor the acetic acid (Periods II and IV) caused any marked increase in the ammonia nitrogen excreted, although it is a little higher in Period IV. The variations in water intake, since this experiment was carried on during the summer, have caused considerable variation in the volume of urine and the analytical data. When the urinary volume, total nitrogen, creatinine, and creatine are plotted on the

TABLE IV.

Urinary Constituents. Daily Average of Last 8 Days of Each Period.

	Period.				
	I, control.	II, lactic.	III, sulfuric.	IV, acetic.	V, control.
Volume, *cc.*	851	575	1,026	994	742
Specific gravity	1.019	1.028	1.026	1.019	1.023
Acidity, 0.1 N, *cc.*	272	292	414	—	—
Total N, *gm.*	10.19	10.28	11.85	11.59	10.62
Urea N, *gm.*	7.15	7.46	5.99	7.48	6.94
NH_3-N, *gm.*	1.50	1.24	4.00	1.85	1.54
Creatinine N, *gm.*	0.311	0.320	0.416	0.341	0.310
Creatine N, *gm.*	0.079	0.102	0.066	0.240	0.148
CaO, *gm.*	0.217	0.232	0.735	0.234	0.108
Total S, *gm.*	0.643	0.609	4.715	0.740	0.679
Sulfate S, *gm.*	0.582	0.550	4.342	0.581	0.584
Unoxidized S, *gm.*	0.060	0.058	0.373	0.145	0.095

same sheet, it is evident that many of the variations in these constituents are due to the changes in the water intake and urine volume. The higher water intake during Period IV thus accounts for a slight increase in urinary ammonia. Daily tests for either lactic or acetic acid in the urine of their respective periods were always negative. It is probable, therefore, that these acids were quite completely oxidized.

In Period III the addition of sulfuric acid to the ration caused a large increase in the urinary ammonia, the greater part of which is compensated for by the decrease in urea excreted. It is noteworthy that the creatinine excretion is somewhat higher, although

McCollum and Hoagland found no increase in the case of a pig on a nitrogen-free diet.[9] It may also be noted that the creatine nitrogen is no higher in Period III than in Period I. The extra creatine excretion in the two organic acid periods may or may not be accidental.

The nitrogen balance for this experiment is given in Table V. It shows plainly that the large amount of sulfuric acid ingested did not interfere with nitrogen storage, as was demonstrated with swine and calves by Steenbock, Nelson, and Hart.[10]

Of the extra acidity ingested, *viz.* 300 cc. normal solution, 184 cc. are accounted for by the increased ammonia excretion. Apparently only 14 cc. are excreted as extra urinary acidity. This leaves about 100 cc. of normal solution unaccounted for. As

TABLE V.

Nitrogen Balance by Periods. Experiment 1.

Period.	N intake.	N in feces (dried).	N in urine.	N Total excreted.	N balance.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
I, control	235.91	65.81	103.66	169.47	+66.44
II, lactic	259.24	71.72	104.54	176.26	+82.98
III, sulfuric	259.24	61.17	113.97	175.14	+84.10
IV, acetic	259.24	57.88	116.09	173.97	+85.27
V, control	259.24	69.34	108.84	178.18	+81.06

suggested in the preceding paper the greater part of this may be excreted in the feces, either combined with bases or in some other form.[11] The calcium balance by periods is shown in Table VI, which apparently accounts for 25 cc. of normal solution of acid daily.

The extra excretion of calcium in the feces in Period III, decreasing the positive balance, may have been due to the formation of calcium sulfate in the intestine during the early stages of digestion, most of which, on account of its relative insolubility, was

[9] McCollum, E. V., and Hoagland, D. R., *J. Biol. Chem.*, 1913–14, xvi, 314.

[10] Steenbock, H., Nelson, V. E., and Hart, E. B., *J. Biol. Chem.*, 1914, xix, 399.

[11] A complete mineral analysis of the feces was planned but could not be carried out, because of present conditions.

excreted as such in the feces. We have already suggested that the neutralization of acid fed by mouth might be more wasteful of fixed base than the neutralization of an equivalent amount of acid formed in normal metabolism, and this observation agrees with that hypothesis. Givens and Mendel[12] found that the administration of 1.5 gm. of hydrochloric acid per day to a dog caused a diversion of calcium from the intestinal path of excretion to the urine, with no change in the calcium balance. Our results (Table VI) show the increase in urinary calcium, but another increase instead of a decrease in the fecal calcium. It is probably due to the difference in solubility between the chloride and sulfate of calcium, and also agrees with our hypothesis. Therefore the loss of calcium shown in the table may be due to the char-

TABLE VI.

Calcium Balance. Experiment 1.

Period.	Ca intake, CaO.	Ca in feces, CaO.	Ca in urine, CaO.	Total excreted.	Balance by periods.	Average daily balance.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
I, control	92.54	57.64	2.07	59.71	+32.83	+3.28
II, lactic	101.70	65.15	2.33	67.48	+34.22	+3.42
III, sulfuric	101.70	69.03	6.89	75.92	+25.78	+2.58
IV, acetic	101.70	63.59	2.30	65.89	+35.81	+3.58
V, control	101.70	67.53	1.37	68.90	+32.80	+3.28

acter of the acid and the method of administration. One would certainly not be justified in assuming that if it were possible to find a wholly satisfactory ration containing a potential mineral acidity equal to the amount of acid we fed, such a ration would cause the decrease in positive calcium balance we have noted here. While the calcium in the ration was not more abundant than animal husbandry experience would justify for practical feeding, it was high enough to cause satisfactory storage of calcium. In our second experiment on a ration low in calcium, the animal was not so wasteful of lime in neutralizing the acid fed, but neutralized a larger proportion with ammonia and phosphates.

It is interesting to note that the lactic and acetic acids in Periods II and IV apparently caused an increased storage of

[12] Givens, M. H., and Mendel, L. B., *J. Biol. Chem.*, 1917, xxxi, 428.

lime. The following explanation seems possible. Perhaps the soluble calcium salts of lactic and acetic acids were absorbed and taken to the tissues where the acid radical was oxidized, thus leaving the calcium in a more favorable position for retention. The fact that during these two periods the urinary calcium was also increased over the control periods also makes it appear that the absorption of calcium from the tract in the form of these soluble salts was increased, with consequent increase both in calcium storage and in urinary calcium.

The hydrogen ion concentration of the blood was again determined on the last day of the sulfuric acid period and found to be 7.2.

Low Calcium Ration.

The object of the second cage experiment was to determine the effect of mineral acid on the calcium balance using a ration very low in calcium, and also to check the urine analysis on a constant water intake. This experiment was carried on in October in a uniformly heated room, and it was intended to allow a definite amount of water throughout the experiment. The feeder failed to allow extra water during the last period to compare with the volume of acid solution given in Period II, and the urine volume dropped unusually low in Period III. More uniform daily results were obtained, however, in Periods I and II.

The ration used was 95 per cent of ground corn and 5 per cent of "black albumin," a blood product low in calcium, but containing over 88 per cent of protein. The calcium content of the mixed feed was 0.022 per cent, and the nitrogen 2.04 per cent, or calculated as crude protein, 12.75 per cent. This was a satisfactory ration except for its very low content of lime.

The analytical methods were the same as used in the first experiment, except that inorganic sulfates were determined instead of total sulfates, which include a small amount of ethereal sulfates. The results of the urine analysis are given in Table VII.

Averages by periods are shown in Table VIII, the first 2 days of each period having been omitted in calculating the averages for reasons previously mentioned.

It will be noted at once that the increase in ammonia excretion is greater than in the first experiment for the same amount of

TABLE VII.

Urine Analysis. Experiment 2.

Period.	Date of sample.	Volume.	Specific gravity.	Reaction to litmus.	Acidity, 0.1 N.	Total N.	Urea N.	NH_3-N	Creatinine N.	Creatine N.	CaO	Inorganic sulfates as H_2SO_4.	Remarks.
	1917	*cc.*			*cc.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
I	Oct. 22	780	1.017	Neutral.	164	8.797	6.118	1.631	0.411	—	0.086	—	Control period.
	" 23	1,145	1.016	Acid.	355	11.301	7.896	1.940	0.513	0	0.098	—	
	" 24	1,160	1.016	"	383	11.433	8.088	1.656	0.536	0	0.118	—	
	" 25	1,050	1.019	"	378	12.524	9.275	1.485	0.526	0	0.086	—	
	" 26	820	1.020	"	271	10.137	8.059	1.217	0.456	0	0.079	—	
Average		991	1.018		310	10.84	7.89	1.59	0.488	0	0.093		
II	Oct. 27	1,440	1.020	Acid.	374	12.197	8.225	2.339	0.618	0	0.317	10.359	Wt. of animal, 137 lbs.
	" 28	1,005	1.030	"	543	10.533	6.228	2.856	0.431	0.007	0.435	14.354	
	" 29	1,320	1.025	"	673	12.567	5.692	4.657	0.661	0.102	0.443	15.114	
	" 30	1,165	1.029	"	804	11.883	5.676	4.632	0.357	0.388	0.475	14.015	300 cc. N H_2SO_4 daily with
	" 31	965	1.030	"	627	12.132	5.500	4.550	0.284	0.568	0.670	13.905	feed.
	Nov. 1	915	1.032	"	540	10.607	5.047	4.074	0.339	0.348	0.587	12.925	
	" 2	1,190	1.024	"	666	12.678	5.964	4.698	0.447	0.149	0.688	13.671	
	" 3	1,155	1.028	"	762	13.405	6.170	4.916	0.358	0.555	0.681	14.550	
	" 4	1,030	1.028	"	670	10.997	4.695	4.557	0.364	0.268	0.611	13.421	
	" 5	1,090	1.029	"	730	13.368	5.890	5.219	0.268	0.460	0.747	15.540	
Average		1,127	1.028		639	12.04	5.91	4.25	0.413	0.285	0.565	13.785	
III	Nov. 6	780	1.028	Acid.	679	14.218	6.813	3.189	0.249	0.337	0.352	4.667	Wt. of animal, 153 lbs.
	" 7	750	1.029	"	660	11.970	7.266	2.352	0.425	0.219	0.188	2.546	
	" 8	590	1.033	"	513	10.209	6.456	1.817	0.296	0.295	0.119	1.882	Control period.
	" 9	585	1.035	"	673	11.029	6.929	1.785	0.288	0.279	0.153	2.038	
	" 10	440	1.036	"	484	8.399	5.605	1.220	0.186	0.192	0.063	1.394	
Average		629	1.032		602	11.17	6.61	2.07	0.289	0.264	0.175	2.505	

acid intake. In the first experiment the per cent of urinary nitrogen as ammonia was 14.7 per cent in the first control period and 33.8 per cent in the sulfuric acid period. In the second experiment the figures were 12.8 per cent in the first control period and 38.2 per cent in the acid period. This may be due to the fact that there was very little calcium available to hold part of the sulfuric acid in the intestine as calcium sulfate. In this experiment 96 per cent of the sulfuric acid ingested was excreted in the urine, while in the first experiment but 90 per cent appeared in the urine, including the ethereal sulfates.

In this experiment there was an apparent decrease in creatinine excretion during the acid period, and a marked increase in

TABLE VIII.

Urinary Constituents. True Daily Averages by Periods. Experiment 2.

	Periods.		
	I, control.	II, sulfuric.	III, control.
Volume, *cc.*	1,010	1,104	538
Specific gravity	1.018	1.028	1.034
Acidity, 0.1 N, *cc.*	377	684	557
Total N, *gm.*	11.37	12.21	9.88
Urea N, *gm.*	8.47	5.58	6.33
NH_3-N, *gm.*	1.45	4.66	1.61
Creatinine N, *gm.*	0.506	0.385	0.257
Creatine N, *gm.*	0.0	0.355	0.255
CaO, *gm.*	0.094	0.613	0.112
Inorganic sulfates as H_2SO_4, *gm.*	—	14.143	1.771

creatine. There are too many other possible factors concerned in the excretion of these substances to allow an explanation with the data at hand.

The extra ammonia excreted during the acid period accounts for 229 cc. of the 300 cc. normal solution of acid ingested. The extra urinary acidity accounts for 31 cc., leaving only 40 cc. not taken care of by these two factors in the urine. The calcium balance data (Table IX) show that the difference is not made up by calcium excretion, although there is a slight increase in the negative calcium balance over the average of the two control periods. The difference between the balances in the two control periods is due to the fact that the experimental animal had been

fed the high calcium ration of our first experiment until 5 days before the beginning of the first control period of this experiment. The preliminary 5 day period was not long enough to get rid of what might be considered excess calcium in the tissues of the animal. Therefore for comparison with the acid period it is necessary in this case to take the average of the two control periods. The negative calcium balance throughout is of course due to the inadequate supply of lime in the ration. It should be noted that the extra calcium excretion in the acid period is very

TABLE IX.

Calcium Balance. Experiment 2.

Period.	Ca intake, CaO.			Ca in feces, CaO.	Ca in urine, CaO.	Total CaO excreted.	Ca balance, CaO.	Average daily balance, CaO.
	Feed.	Water.	Total.					
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
I, control, 5 days....	1.50	0.83	2.33	5.47	0.46	5.93	−3.60	−0.72
II, H_2SO_4, 10 "	3.00	1.56	4.56	5.84	5.65	11.49	−6.93	−0.69
III, control, 5 "	1.50	0.79	2.29	2.12	0.88	3.00	−0.71	−0.14

TABLE X.

Nitrogen Balance. Experiment 2.

Period.	N intake.	N in feces (dried).	N in urine.	Total N excreted.	N balance (by periods).	Average daily N balance.
	gm.	gm.	gm.	gm.	gm.	gm.
I, control, 5 days.....	138.92	22.13	54.19	76.32	+62.60	+12.52
II, H_2SO_4, 10 "	277.85	30.98	120.37	151.35	+126.50	+12.65
III, control, 5 "	138.92	18.70	55.82	74.52	+64.40	+12.88

small, and that on a low calcium ration the animal was much more economical with the calcium supply than in the first experiment. Even on the high calcium ration, however, only 5 per cent of the very high acid intake was apparently neutralized by calcium, and this may have been largely due to the insolubility of the salt which was the product of neutralization. Although stress has been laid on the effect of natural acid rations on the skeleton, an inspection of the two calcium balance tables shows that the loss caused by the very high amount of acid we fed was

not in either case serious, especially in the first experiment, when the ration was adequate to meet the normal needs of the growing animal.

The nitrogen balance is shown in Table X. As in the first experiment there was certainly no interference by the acid with nitrogen storage.

We intend to continue this work later, to determine, if possible, the fate of the fraction of the acid not yet accounted for.

SUMMARY.

Metabolism studies on a growing pig were conducted to secure definite information regarding the fate of ingested lactic, acetic, and sulfuric acids in the animal body.

On a ration containing a liberal allowance of calcium it was found that the animal apparently oxidized the organic acids completely, with no increase in urinary ammonia, and that they seemed to bring about a slightly increased retention of calcium.

On the same basal ration plus 300 cc. of normal sulfuric acid per day, 61 per cent of the acid ingested was neutralized by means of ammonia, and 5 per cent excreted as phosphates. On another basal ration very low in calcium, extra ammonia excretion accounted for 76 per cent of the acid fed, and extra urinary acidity for 10 per cent. On neither ration did the mineral acid cause a significant loss of calcium, nor did it interfere with the storage of protein.

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CONTENTS

MORGAN, AGNES FAY, and HEINE, ALICE M. Biological values of wheat and almond nitrogen 215

OSBORNE, THOMAS B., MENDEL, LAFAYETTE B., and FERRY, EDNA L. A method of expressing numerically the growth-promoting value of proteins 223

MORRIS, J. LUCIEN. New titration method for the determination of uric acid in urine .. 231

MYERS, VICTOR C., and FINE, MORRIS S. Comparative distribution of urea, creatinine, uric acid, and sugar in the blood and spinal fluid 239

DENIS, W., and MINOT, A. S. Creatinuria and acidosis 245

GIVENS, MAURICE H., and MCCLUGAGE, HARRY B. The antiscorbutic property of vegetables. I. An experimental study of raw and dried tomatoes 253

PALMER, LEROY S., and SCOTT, ROBERT G. The physicochemical state of the proteins in cow's milk 271

VAN SLYKE, L. L., and BOSWORTH, A. W. The state of proteins in cow's milk 285

MCCOLLUM, E. V., SIMMONDS, N., and PARSONS, H. T. The dietary properties of the pea (*Vicia sativa*) 287

HASKINS, HOWARD D. Modifications of Benedict's and Folin's quantitative sugar methods 303

ROHDE, ALICE, and STOCKHOLM, MABEL. The increase in nitrogen metabolism of the dog, following the administration of desiccated thyroid gland 305

LAMB, ALVIN R., and EVVARD, JOHN M. The acid-base balance in animal nutrition. I. The effect of certain organic and mineral acids on the growth, well being, and reproduction of swine 317

LAMB, ALVIN R., and EVVARD, JOHN M. The acid-base balance in animal nutrition. II. Metabolism studies on the effect of certain organic and mineral acids on swine. 329

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